AD_____

STUDY OF THE EFFECTS OF DRUGS UPON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

ANNUAL PROGRESS REPORT

by

Robert W. Caldwell

Clinton B. Nash

June 1, 1986

(January 1, 1985 - APRIL 15, 1986)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

FORT DETRICK, FREDERICK, MARYLAND 21701-5012

Contracts No: DAMD17-83-C-3011

University of Tennessee Center for the Health Sciences
Memphis, Tennessee 38163

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During this past year we have: 1. Completed study of Determination of the Involvement of Histamine in the Blood Pressure Response to Liposome. A copy of this report is attached (Section I). 2. Written a protocol to study the Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog.: Correlation with Blood Cholinesterase Inhibition. A copy of this protocol (originally submitted on 19 June, 1985) is attached (Section II). 3. Completed study of the Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition. A copy of this report is attached (Section III).

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SUMMARY

During this past year we have:

1. Completed study of Determination of the Involvement of Histamine in the Blood Pressure Response to Liposome. A copy of this report is attached (Section I).

2. Written a protocol to study the Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition. A copy of this protocol (originally submitted on 19 June, 1985) is attached (Section II).

3. Completed study of the Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition. A copy of this report is attached (Section III).

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised, 1985).

Table of Contents

SECTION I.

- LETTER REPORT -

DETERMINATION OF THE INVOLVEMENT OF HISTAMINE IN THE BLOOD PRESSURE RESPONSE TO LIPOSOME

Introduction

Liposome carrier suspension produces an arterial hypotension when given intravenously. In our recent studies, prior treatment with compound WR-149, 024, reported to stabilize histamine-containing cells and antagonize factors which induce histamine release, appeared to reduce this hypotension (June, 1984). These findings have led to the speculation that histamine is involved in this hypotensive response.

Purpose

If release of histamine from body stores is responsible for the hypotension to liposome injections, we reasoned that a tachyphylaxis should develop as histamine stores are depleted. In dogs with chronically indwelling catheters to measure systemic and pulmonary arterial pressures, we wished to determine if repeated i.v. bolus injections of a given amount of liposome carrier suspension would yield systemic or pulmonary arterial pressure responses which are progressively smaller. The purpose of this study was to determine the effects of carrier liposome suspension upon histamine release by observing arterial blood pressure changes and analyzing plasma samples.

Methods

Two male beagles were anesthetized with xylazine (2.2 mg/kg, i.m.) and halothane and chronically fitted with both a Swan-Ganz balloon-tipped catheter with features for entry into the pulmonary artery and polyethylene cannula (PE 260) into the femoral artery to the level of the abdominal aorta. Dogs were maintained on 100 units/kg/day Na-heparin (s.c.) to prevent thrombosis. After a seven-day recovery period, the animals were anesthetized with Na-pentobarbital (30 mg/kg, i.v.) and allowed to reach a steady level of anesthesia. Measurement of pulmonary arterial pressure was made through the Swan-Ganz catheter. Systemic arterial blood pressure was obtained via the Femoral arterial cannula externalized to the nape of the neck. Control values were obtained for pulmonary artery pressure and arterial blood pressure. Bolus i.v. doses of 1.0 - 5.0 ml of liposome carrier were administered over a 2-4 second interval directly into the right cardiac ventricle via the proximal port of the Swan-Ganz catheter. Venous blood samples (approximately 5 ml each) were taken just before injection of the liposome suspension and at the nadir of the arterial pressure response (or 5 min post injection if no response occurred); plasma was immediately acidified (pH=4-5) with HCl and frozen. Samples may be shipped to Burroughs-Wellcome for histamine analysis.

Results

Volumes of 1, 2, and 3 ml of the liposome suspension did not elicit any systemic or pulmonary pressor responses in either of the two beagles. A total of ten injections were given over a series of three separate experiments. Experiment 2-8-85 (performed with beagle #1) included two 1 ml doses and one each of 2 and 3 ml. Experiment 2-19-85 (performed with beagle #2) included one dose each of 1, 2, and 3 ml. Experiment 2-22-85 (performed with beagle #2) included two 2 ml doses and one 3 ml dose.

However, in a fourth experiment (2-26-85, performed with beagle #2) 2 separate volumes of 4 and 5 ml caused a marked systemic (15 and 35 mmHg, respectively) and pulmonary (9 and 5 mmHg, respectively) hypotension. A test dose of a comparable volume of saline into the right cardiac ventricle gave no response (See figures 1a & 1b). Blood plasma samples have been taken before and after each injection of liposome suspension and may be sent, wholely or in part, to Burroughs-Wellcome for analysis of histamine.

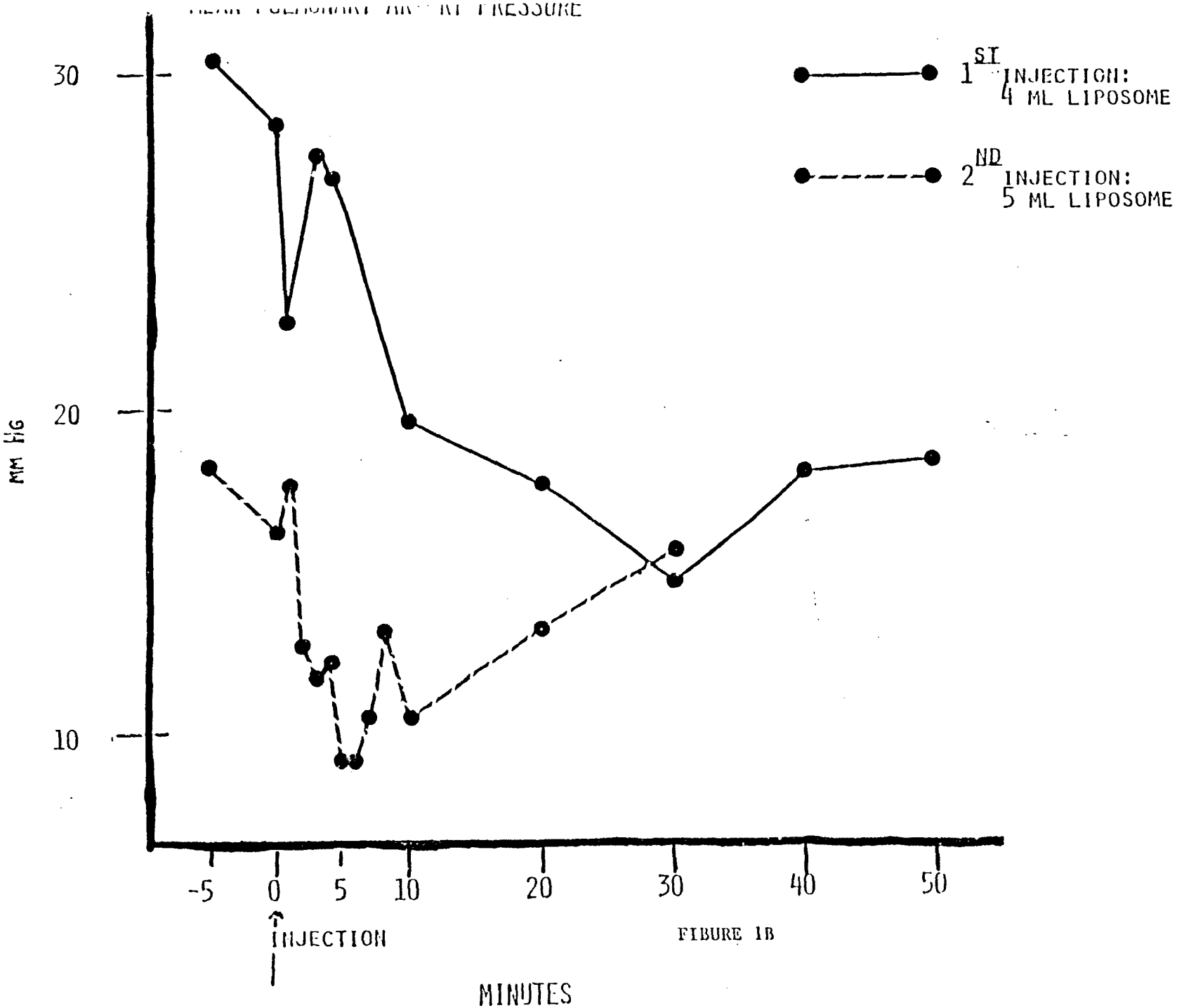
Summary and Conclusions

Bolus i.v. injection 1, 2, and 3 ml of liposome into each of two anesthetized beagles did not produce a change in arterial pressure. However, in one beagle, volume of 4 and 5 ml of liposome carrier produced a systemic and pulmonary hypotension. therefore, relatively large volumes of liposome suspension injected as a bolus are required to reduce arterial blood pressure. The fact that injection of liposomes causes a fall in pulmonary artery pressure is not entirely consistent with the concept of histamine release by liposomes, since histamine is a vasoconstrictor in the pulmonary vascular bed of the dog. However, if the site of histamine release is passed (the pulmonary resistance vessels), enough histamine may not reach the pulmonary vessels through return of blood to produce vasoconstriction. The fall in pressure in the pulmonary circuit might be passively due to a transient reduction in venous return.

Robert W. Caldwell, Ph.D.

Clinton B. Nash, Ph.D.

Terrye R. Thomas, B.S.


MEAN PULMONARY ARTERIAL PRESSURE
30
20
10
MM HG
1ST INJECTION:
4 ML LIPOSOME
2ND INJECTION:
5 ML LIPOSOME
-5
0
5
10
20
30
40
50
INJECTION
MINUTES

FIBURE 1B

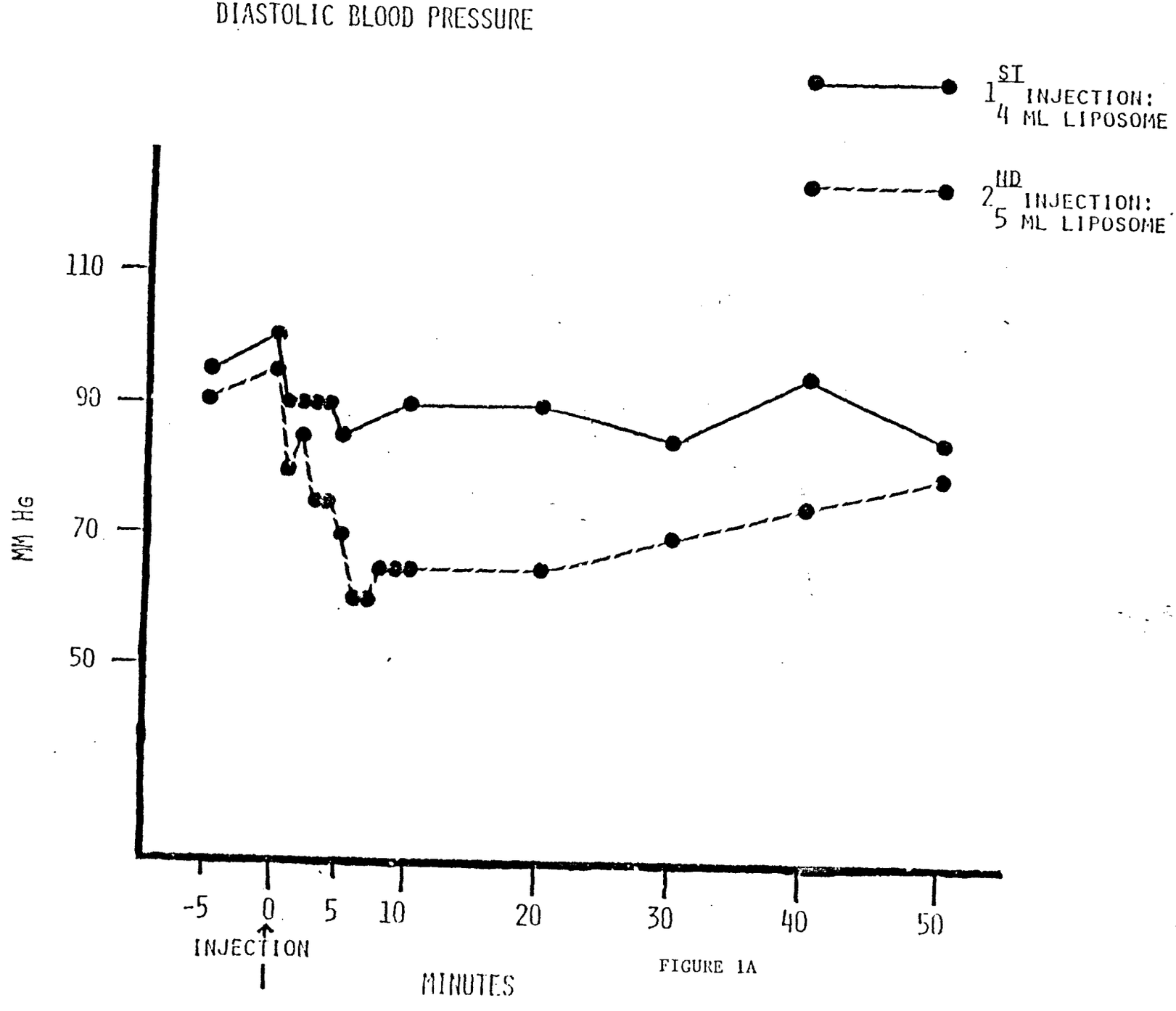

DIASTOLIC BLOOD PRESSURE
SI
1 INJECTION:
4 ML LIPOSOME
HD
2 INJECTION:
5 ML LIPOSOME
110
90
70
50
MM HG
-5
0
5
10
20
30
40
50
INJECTION
MINUTES

FIGURE 1A

PROTOCOL FOR STUDY

Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition

Robert W. Caldwell
Clinton B. Nash
Terrye R. Thomas
Cheryl Rieck

Department of Pharmacology
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Memphis, TN 38163

Contract/DAMD 17-83-C-3011

U.S. Army Medical R&D Activity

19 June 1985

(Revised 27 August 1985)

INTRODUCTION

Pyridostigmine Bromide is a reversible inhibitor of acetylcholinesterase. This agent increases plasma and tissue half-life and the biological effects of acetylcholine. The main therapeutic use of this agent is for management of myasthenia gravis, a disease manifested by skeletal muscle weakness resulting from reduced density of acetylcholine receptors at the neuromuscular junction.

A recent report has given information about the cardiopulmonary actions of pyridostigmine Br in conscious dogs (Ehrlich et al, 1985). At a dose of 1 mg/kg (i.m.) pyridostigmine increased respiratory rate and minute volume as well as airways resistance, in resting dogs. Heart rate, cardiac output, and blood pressure were not altered. In exercising dogs, respiratory rate increased, but minute volume and airway resistance were unchanged. Heart rate was lowered, but cardiac output was not changed. Pulmonary artery pressure was elevated in both resting and exercising animals. Fifteen minutes after injection (1 mg/kg, i.m.), a steady state plasma cholinesterase level of 40% of control was reached.

Our proposed studies would determine and correlate the cardiovascular and pulmonary actions of graded intravenous doses of pyridostigmine Br in the anesthetized dog. The range of doses to be studied would be from that producing 1) a small but definite effect upon cardiovascular and/or pulmonary function, to a dose which produces 2) severe changes in cardiopulmonary function, just short of death. A dose producing effects intermediate to these

---

[1]Ehrlich W.; Jayaweerce, A.R.; Gruilarte, T.R.; Bassett, D.J.P.; and Abbey, H. The Effect of Pyridostigmine Injections on Vital Functions of Dogs at Rest and during Exercise. Annual Mtg. - U.S. Army Med. R&D Command 5th Ann. Chem. Def. Bios. Rev. May, 1985.

extremes will also be used. In addition, these studies would correlate plasma acetylcholinesterase activity with cardiovascular and pulmonary function before, during and after administration of pyridostigmine. The formulation of this drug to be utilized is Mestinon® sterile solution (5 mg/ml).

Our Preliminary Studies: In initial range finding studies with over 12 anesthetized mongrel dog preparations, we have found that a total dose of 5 mg/kg (i.v.) of pyridostigmine (Mestinon®) appears to be the maximum tolerated. Higher doses of 6 and 7 mg/kg produces death by apparent respiratory paralysis in 2 of 3 dogs tested. A dose of 0.5 mg/kg appears to be the lowest dose which consistantly produces notable changes in cardiovascular and pulmonary function. A dose of 2 mg/kg produces intermediate effects.

For doses of 5 to 0.5 mg/kg of pyridostigmine, delivered over a 15 minute period, the most evident changes involve respiratory variables and P-R interval. Changes in arterial pressure are slight and a moderate rise has been noted in pulmonary artery pressure. Tidal volume is reduced but respiratory rate is increased; as a result, minute volume in most of the animals tends to be maintained. Airways resistance shows a very pronounced dose-related increase, while airways compliance is reduced. Heart rate is slowed and P-R interval is lengthened. Following the acute effects during and just after drug infusion, values for these variables return toward control levels.

Whole blood cholinesterase activity is reduced by pyridostigmine over the 0.5 to 5 mg/kg dose-range. The lowest level of activity occurs at the end of the 15 minute infusion; cholinesterase activity has recovered only slightly by +25 minutes, remaining at this depressed level throughout the remainder of the 120 min post-infusion observation period. The maximum depression of enzyme activity, which occurs at +15 min, is approximately 30, 55, and 80%, for 0.5, 2 and 5 mg/kg respectively.

Because of a new Department of Army policy which forbids the use of pound dogs in sponsored research, we have recently performed this same experimental procedure in pure-bred beagle dogs. To date, eight experiments have been completed.. Our data indicate that the cardiopulmonary responses of beagles to pyridostigmine are similar to those obtained in mongrels.

METHODS

I. Drug Preparation and Delivery

Pyridostigmine Br (mw = 261.14), 1-methyl-3-hydroxypyridinium bromide dimethylcarbamate, purchased from Roche Laboratories as Mestinon® [Lots 0101 and 0102 for injection, 5 mg/ml, 2 ml/ampul]. will be diluted fresh daily for each experiment with normal saline to an appropriate concentration to deliver the desired dose in a volume of 29 ml (1.91 ml x 15 min). Aliquots of the vial contents will be accurately weighed to produce solutions for infusion.

II. Animals

In conducting these studies the investigators will adhere to the "Guide for the Care and Use of Laboratory Animals", DHEW Publication 78-23, Reprinted 1980. Beagle dogs of either sex, 9 months and older, weighing between 9.0 and 14.0 kg will be purchased from Riglan Animal Care Systems (Mt. Horeb, WI) for these studies. The dogs are guaranteed to be in excellent health and filaria free. One ml of blood will be taken and checked for the presence of microfilaria using the Knott's test, an indicator of possible worm infestation, before onset of the experiment. Also, only dogs with normal ECG profiles will be used. Dogs will be anesthetized with pentobarbital Na, 30 mg/kg, intravenously and maintained with supplemental injections of 1.0 mg/kg as necessary to maintain stable anesthesia. Corneal and plantar reflexes, response to pain, and respiratory rate (16-20 breaths/min) will be used in "titrating" the dog to the desired level of anesthesia.

III. Cardiovascular Measurements

N.B. See appendix for details of instrument calibration procedures.

All catheters for pressure measurements will be filled initially with heparinized saline (30 units/ml) and flushed with heparinized saline as needed to maintain patency. A femoral artery will be catheterized with polyethylene

tubing (PE 260) advanced to the thoracic aorta and connected to a Statham P23AC pressure transducer for measurement of arterial blood pressure. The left carotid artery will be cannulated with a Millar® Mikro-tip® catheter pressure transducer (Model PC-350, size 5F) for high fidelity measurement of left ventricular pressure (LVP). The rising slope of the LVP will be differentiated to give dP/dt, an estimate of myocardial contractility. The left ventricular pressure signal will also be used to record left ventricular end diastolic pressure. Heart rate will be recorded via a Grass cardiotachometer triggered by the R wave of the Lead II EKG. Short strips of standard leads will be recorded every 10 minutes and more frequently as required during infusion at 25 mm/sec and Lead II rhythm strip will be recorded every 10 minutes, or as required, at 100 mm/sec.

One femoral vein will be catheterized to the level of the heart for drawing venous blood samples (approx. 4 ml) and the other femoral vein will be cannulated for drug infusion. Arterial blood samples (approx. 5 ml) will be drawn from the aortic pressure line at selected time points. Samples will be taken in glass syringes rinsed with heparinized saline following evacuation of the static volume in the cannulae. Syringes containing blood will immediately be capped with tight-fitting rubber nipples and put in ice until analysis. The first samples drawn will be designated as -15 minutes. At time 0, just prior to onset of the infusion of drug or vehicle, another set of blood samples will be taken. Additional sets of blood samples will be taken at +7.5, +15, +25, +45, +85, and +115 minutes. One ml of arterial blood will be transferred into a glass test tube rinsed with heparinized saline, frozen to lyse the cells, thawed, and used for analysis of cholinesterase activity at -15, 0, +15, +25, +45, +85, and +115 minutes. Microhematocrit will be assessed using

venous blood. Withdrawal of blood will be performed by a person other than the operator of the blood gas analyzer.

Blood Analyses

All samples will be analyzed for pH and dissolved O_2 and CO_2 by Cheryl Reick using the Corning Model 165/2 Blood Gas Analyzer within 10 minutes. Each sample (4 ml each of arterial and venous blood) will be analyzed in triplicate. All analyses will be performed according to the procedures published by the company for this analyzer. Calibrations will be made with standard gas mixtures and pH buffers.

Assay of whole blood cholinesterase will be performed as described by Ellman et al. (1961)[2] and Siakotos et al. (1969)[3]. Either fresh or frozen blood (1-4 days) will be used; tests in our laboratory have shown that there is no difference in results obtained from blood handled in either manner. Briefly, this method for assessing total whole blood cholinesterase activity involves the 20 min incubation of 10µl of bood within 300 µl of a buffer-salt-detergent solution containing [acetyl-^{14}C]-choline Iodide (New England Nuclear ~4.4 mCi/mmol) and cold acetylcholine at a concentration of 10^{-3}M ($6X10^6$ cpm/ml). The reaction will be stopped by addition of a suspension of Amberlite IRP-69 resin in dioxane. This resin binds unhydrolyzed (intact) acetylcholine. The resultant mixture will be centrifuged (900 g for 1 min) and an aliquot of the supernatant solution containing free ^{14}C-acetate measured by a liquid scintillation counter. Non-enzymatically produced free ^{14}C-acetate will be determined by assay of reaction mixture containing water rather than plasma.

Cardiac Function

For the measurement of cardiac output an Instrumentation Laboratories thermal dilution cardiac output computer (see appendix for theory of opera-

tion) will be used. A Swan-Ganz type catheter (Swan et al., 1970) with an extra injection port and a temperature sensor will be connected to a Statham P23Gb pressure transducer (ultra low volume displacement) and will be inserted into the right jugular vein. The balloon at the catheter tip will be inflated and the catheter "floated" through the right atrium and the right ventricle into the pulmonary artery. The distinctive changes in pressure tracings will easily identify these locations. The balloon tip is advanced into the pulmonary artery until an abrupt drop in pressure and cessation of pulsation indicate that the balloon has occluded the pulmonary artery. The balloon is then deflated (restoring the pressure pulsations) and the catheter advanced a few more mm, maintaining the normal pulmonary artery pressure pulsations. The catheter is then securely tied in place. Upon re-inflation of the balloon tip (for 15 sec), pulmonary wedge pressure (an indication of left atrial pressure) will be recorded at desired time points. With the balloon deflated, pulmonary artery pressure will be continuously recorded. Cardiac output will be determined from this same catheter. Outputs are obtained in duplicate at given time points by injection of 3 ml of saline at zero degrees C and a subsequent computer integration of the temperature change vs time curve as detected by the thermistor. The two determinations for each point will be taken at the same place in the respiratory cycle and averaged.

Cardiovascular variables will be recorded on both a Grass Model 7B polygraph and a Texas Instruments Model 675 data terminal. Pulmonary Vascular Resistance (PVR) will be calculated according to the formula: PVR = PAP - LVEDP/CO, where PAP = pulmonary artery pressure, mm Hg; LVEDP = left ventricular end diastolic pressure, mm Hg; CO = cardiac output or pulmonary artery blood flow, L/min. Interpretation of the drug's action must take into account possible flow-induced changes in the pulmonary vasculature.

IV. Pulmonary Ventilatory Measurements

N.B. See Appendix for instrument calibration procedures and details of pulmonary ventilatory measurements.

For the measurement of pulmonary function while breathing room air unassisted, an endotracheal tube with a side arm will be connected directly to a mesh screen Fleish pneumotachograph and the pressure difference across the screen measured by a differential pressure transducer (Validyne® transducer Model MP45-24). This signal, when calibrated against a known air flow, corresponds to tidal airflow rate and, in turn, when integrated, yields tidal volume. Also, an esophageal tube (Porter ID 6.5) will be inserted into the esophagus for the assessment of intrapleural pressure. The pressure difference between the airway and esophagus, or transpulmonary pressure, will be measured by a second differential pressure transducer (Validyne transducer Model MP45-14). Dynamic airway resistance ($\Delta P/\Delta F$) and dynamic airway compliance ($\Delta V/\Delta P$) will be computed using a Buxco Electronics Pulmonary Mechanics Computer Model 6. Airway resistance and compliance and tidal volume will be recorded as analog signals with a Grass Model 7b polygraph and as digital values with a Texas Instruments Model 765 data terminal.

V. Other Observations

Because this particular drug often causes increased mucous secretion, the volume of mucous produced and released about mouth and nose will be estimated by the use of a funnel and graduated cylinder under the dog's snout. Following euthanasia with KCl, the lungs will be inspected grossly for abnormalities and a lung lobe will be tied off and placed in water for an estimate of density. Inspection of the heart and lungs will verify absence of filaria.

Also, because pilot studies showed that this drug seems to induce defecation, frequency of defecation will be noted.

VI. Data Presentation

All measurements called for in this protocol will be presented in tabular form using percent change of baseline where Baseline = 100% (unless otherwise indicated)[4]. Summary graphs will be constructed to show percentage change from baseline (or control) which will be considered as 100%. Variability for each measurement will be given as ± 1 S.E.M. for mean percent of baseline (unless otherwise indicated). For purposes of clarification, several related variables will be plotted on a single graph. In addition, samples of actual tracings will be used to illustrate typical responses to various doses.

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2. Ellman, George L.; K.D. Courtney; V. Andres, Jr. and R.M. Featherstone. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochem. Pharm. Vol. 7, pp. 88-95, 1961.
3. Siakotos, A.N.; M. Filbert and R. Hester. A Specific Radioisotopic Assay for Acetylcholinesterase and Pseudocholinesterase in Brain and Plasma. Biochem. Med. 3:1-12, 1969.
4. Absolute values for all measurements at time zero (baseline) will be given for all groups (mean value ± 1 SEM).

PROPOSED STUDIES

We will use the following protocol and experimental scheme: Approximately 60 minutes will be required following induction of anesthesia to perform the necessary surgery, cannulation procedures, and to establish calibrations. This will be followed by a stabilization period of 20 to 30 minutes to insure that all recordings are steady, and this, in turn, will be followed by a control period of 15 minutes during which data will be recorded. The drug infusion is then begun and continued for 15 minutes. There will be a post-infusion period of 120 minutes for observation of recovery.

I. Observations - 15 minute control period

A. Cardiovascular Measures

1. arterial blood pressure - continuous
2. left ventricular pressure - continuous
   a. dP/dt - continuous
   b. left ventricular end diastolic pressure - continuous
3. electrocardiogram - Lead II, strips recorded at 25 and 100 mm/sec for analysis (-15 and 0)
4. heart rate - continuous; by cardiotachometer
5. pulmonary vascular
   a. pulmonary artery pressure - continuous
   b. pulmonary wedge pressure - at 15 minute interval (-15 and 0)
   c. cardiac output - at 15 minute interval (-15 and 0)
   d. pulmonary vascular resistance - calculated for the 15 minute interval (-15 and 0)

B. Pulmonary Ventilatory Measures

1. Airways differential pressures

a. air flow - signal integrated by preprogrammed Buxco computer

b. transpulmonary pressure (bronchial vs esophageal) - signals utilized by preprogrammed computer

2. Airway integrated measures - tidal volume and minute volume - continuous

3. Airways computer measures

a. compliance - continuous = $\Delta V/\Delta P$

b. resistance - continuous = $\Delta P/\Delta F$

4. Respiratory Rate - continuous

C. Hematological Measures (at -15 and 0)

1. Blood P_{O_2} - arterial and venous (in triplicate)

2. Blood P_{CO_2} - arterial and venous (in triplicate)

3. Blood pH - arterial and venous (in triplicate)

4. Microhematocrit - venous (in duplicate)

5. Cholinesterase activity (as nmoles Acetylcholine hydrolyzed/ml plasma/hr) - arterial (in triplicate)

II. **Drug Infusion for 15 minutes - Observations as described in I: A, B, and C (drug infusion time = 0 - +15 minutes)**

A. Measures A. and B. from I, plotted at +7.5 and +15 minutes with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, and 4 from I.C. at +7.5 and +15 minutes; measure 5 from I.C. at +15 min

III. **Observation period - 120 minutes post-drug**

A. Measures A. and B. from I., plotted at 10 minute intervals beginning at +25 min and continuing through +135 min - with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, 4, and 5 from I.C. taken at +25, +45, +85, and +115 min

EXPERIMENTAL GROUPS

	GROUP	DOSE		NUMBER
1.	Pyridostigmine Br	0.5 mg/kg	(1.9 µmoles/kg)	6 dogs
2.	Pyridostigmine Br	2 mg/kg	(7.7 µmoles/kg)	6 dogs
3.	Pyridostigmine Br	5 mg/kg	(19.2 µmoles/kg)	6 dogs
4.	vehicle* (control)			6 dogs

All doses will be given in a total volume of 29 ml given at a volume-rate of 1.9 ml/min for 15 mins. The cannula will be loaded with drug prior to onset of the infusion to insure accurate drug delivery. Dilution of the Mestinon® will be determined gravimetrically and adjusted with normal saline to deliver the desired dose at the set infusion rate.

*Vehicle control for Mestinon® will be normal saline.

Robert W. Caldwell, Ph.D.
Professor of Pharmacology
Principal Investigator
19 June 1985

C.B. Nash, Ph.D.
Professor of Pharmacology

K. U. Malik, Ph.D.
Quality Control Officer

The conduct of these studies shall comply with the GOOD LABORTORY PRACTICES (GLP) regulations as published in the Federal Register, Volume 43 (247), 22 December 1978, Part II, pp 59,986-60,020 (and all subsequent addenda) as per Task Order. The contracter shall notify the contracting officer (301) 663-2987 and the COTR (302) 427-5148 by telephone immediately upon announcement by a representative of the FDA of an inspection of studies performed under this contract. In addition to the FDA representative, the USAMRDC-appointed COTR shall have access to the contractor's records. With reference to paragraph 58,195(g) of the GLP regulations, the contractor shall notify the contracting officer in writing, in addition to the FDA, should the contractor go out of business and/or transfer the records during the periods prescribed in paragraph 58,195. On expiration or termination of the contract, the contractor shall notify the contracting officer of any remaining unused test articles.

APPENDIX

INSTRUMENT CALIBRATION PROCEDURES

1. Calibration procedure for Statham Pressure Transducers (Model P23AC)

At regular intervals each transducer is connected to the channel of a Model 7b Grass recorder where it will be used and tested for maintenance of calibration. A mercury manometer is inserted in line to the transducer and a known pressure introduced. The excursion recorded is compared to the Grass internal calibration standard of 100 mmHg = 2 cm at sensitivity 10. For each experiment, the recorder sensitivity is set to match the excursion given by 100 mmHg pressure change.

2. Calibration procedure for Buxco Pulmonary Mechanics Computer Model 6 (further explained in instruction book provided with computer)

With power switch in "on" position enter a known flow into the system. 50 ml/sec flow (determined by a Gilmont air flowmeter, size #3, calibration curve provided, connected to a Fleish pneumotachometer and a Validyne differential pressure transducer Model MP45-24) should provide a 10 mm deflection at flow gain setting 5. With power switch in "cal" position, calibration pots are adjusted to a 20 mm deflection. Examine the calibration tidal volume signal, removing notches using fine flow zero. Set tidal volume deflection to 25 mm using TV gain.

With power switch in "on" position enter a given pressure in cm of HOH using a HOH manometer into a closed tube system connected to a Validyne differential pressure transducer, model MP45-149. A pressure of 5 cm HOH = 5 mm excursion (adjust with pressure gain). With power switch in "cal" position, use "cal" pots to set a calibration signal deflection of 10 mm.

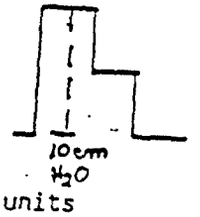
Using the given flow and pressure and employing Program 1 of the computer (see instruction book) we obtain the following:

$$\text{Flow} = \frac{100 \text{ ml/sec}}{100 \text{ ml/sec}} \quad \frac{\text{ins}}{\text{exp}}$$

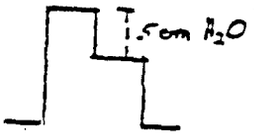
$$\text{Pressure} = \frac{5 \text{ cm } H_2O}{5 \text{ cm } H_2O} \quad \frac{\text{1st plateau}}{\text{2nd plateau}}$$

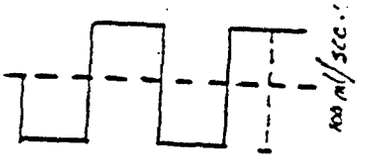
TV = 50 ml/25 mm

Compliance = 50 ml/10 cm H_2O or 5 compliance units



Resistance = 5 cm H_2O/.1 L/sec or 50 resistance units





3. Calibration of the Buxco Data Logger Model DL-12

The Buxco Data Logger Model DL-12 accepts up to twelve analog input parameters, digitizes each, and prints out a value for each parameter at the end of a selected interval. Values contain a maximum of four decimal digits with a decimal point; each represents the average level of a particular parameter over the interval. Each input parameter has a fine gain control associated with it so that each value printed is a direct reading in units of the particular parameter.

Each channel may be calibrated to the unit of measure being sampled. A known input voltage will generate a number up to four digits in size. The user can adjust the generated number to equal the actual value of the parameter sampled (magnitude is determined by the decimal point placement assigned to that channel). This equivalence is accomplished by adjusting the turnpot associated with the channel currently sampled. The user adjusts the turnpot until the terminal displays the desired number. Using a gain factor of one, the largest number which can be generated by the Data Logger is 4095. Gain factor of two can generate a value up to 8190, etc.. The largest gain factor which may be used is nine. A DC input of four volts will generate the maximum value if the trimpot is turned clockwise to the fullest extent.

Calibration signals generated by the Pulmonary and Cardiovascular Analyzers will be set to the following values on the Data Logger:

Channel	Parameter	Value
1	Flow	200 ml/min
2	Pressure	10.0 mmHg
3	Tidal Volume	200 ml/min
4	Compliance	20.0 CU

5	Resistance	12.5 RU
6	Respiratory Rate	15.0 breaths/min
7	Minute Volume	3.0 L/min
8	mean Pulmonary Artery Pressure	20.4 mmHg
9	Heart Rate	120 beats/min
10	Systolic Blood Pressure	150 mmHg
11	Diastolic Blood Pressure	90 mmHg
12	Left Ventricular dP/dt	2000 mmHg/sec

The absolute values are printed out at the end of each interval. The user may select one of the following intervals for Timewise sampling (we will use 1 minutes interval sampling):

1) .1 - 60 samples/6 sec interval

2) .2 - 120 samples/6 sec interval

3) 1.0 - 600 samples/6 sec interval (samples printed at 60 sec intervals)

Note that each parameter is sampled at a rate of ten samples per second, regardless of the type of sampling being performed.

The user may select a set of data values printed out at the end of an interval to be used as the set of "Control" values. To use the Control Value Set Select command, the user simply depresses the alpha key "C" immediately after the chosen line of values is printed out. This line of values is used as the set of "Control" values. The character "CV" appears in the left margin of the logging report on the line immediately following the set of values chosen. The data logger will also compute absolute change and percent change as 100% of "Control" baseline values upon command.

4. Millar® Catheter-tip Transducer

On each experimental day, the transducer catheter to be used is connected through a transducer control unit (Model TCB-10) to a Grass Model 7b Recorder. The reference bridge within the Control Unit is balanced to an original transducer zero pressure baseline. The calibration signal provides transducer bridge excitation voltage in 20 mm steps up to 100 mmHg. We have found the internal calibration signal to be identical to externally applied pressures in a mercury column. We have compared arterial pressures taken simultaneously with the Millar transducer and the Statham transducer and have found them to be the same; furthermore, they remain the same for at least 3 hours. We will calibrate the Millar transducer at the beginning and again at the end of each experiment by putting a known pressure of 100 mmHg on the transducer from a Hg manometer and confirm that the internally generated calibration signal exactly matches the response to the 100 mmHg of pressure. At any time during the experimental procedure, the reference position of the control unit may be used to reproduce the original transducer baseline.

5. Calibration of Cardiac Output System - IL801

The thermal dilution method is based on the proven concept of the Fick principle wherein the dilution of temperature (or dye) is measured by a sensor located downstream and the rate of dilution is calculated. A computer determines the area of a time-temp plot and converts this to cardiac output. The Instrumentation Laboratories cardiac output computer is factory calibrated. The instruction booklet furnishes calibration curves for temperatures and volumes of saline injected. A "K" calibration value is extrapolated. The IL801 is equipped with a Self Test Routine which simulates a 5.0 L/min ± 4% cardiac output (4.80 to 5.20 l/min). Hard copy of the data is provided by

means of the IL801 printer. A Self Test result of 5.0 L/min ± 4% confirms electronic integrity and the operator is then ready to begin meaurements. The catheter itself is fed into the jugular vein and advanced to the pulmonary artery using pressure signals obtained via a Statham P23AC transducer recorded on a Grass polygraph (Swan et al., 1970). The injection port is located 12.5 cm from the sensor tip, designed to inject the 0°C saline into the right ventricle, allowing mixing to occur.

STUDY REPORT
Number 9

CARDIOVASCULAR AND PULMONARY EFFECTS OF INTRAVENOUS PYRIDOSTIGMINE BROMIDE INFUSION IN THE DOG; CORRELATION WITH BLOOD CHOLINESTERASE INHIBITION

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SUMMARY

A range of doses of pyridostigmine were infused intravenously into anesthetized beagle dogs to determine the minimal effective and the maximum tolerated doses upon cardiovascular and pulmonary function and blood cholinesterase (Che) activity. Doses of 0.5 and 5.0 mg/kg infused over 15 minutes were found to fulfill these criteria; a dose of 2 mg/kg produced intermediate effects. The 0.5 mg/kg dose of pyridostigmine produced a slight (10%) fall in tidal volume and airways compliance, a 200% increase in airways resistance, but no change in respiratory rate or minute volume. This dose also produced a 10% fall in heart rate, a small increase in cardiac output (8%), stroke volume (20%) and pulmonary artery (15%) and wedge pressure (2 mmHg), and a 35% fall in blood Che activity. The 5 mg/kg dose produced a 30% fall in tidal volume, a 40% fall in airways compliance, a 1000% rise in airways resistance, a 100% rise in respiratory rate, and a 30% rise in minute volume. This dose also produced a rise during infusion and subsequent fall in systolic pressure, a 30% fall in diastolic pressure, a 45% fall in heart rate, a 25% increase in cardiac contractility, an 80% rise in stroke-volume, a rise and fall in cardiac output, an increase in pulmonary vascular resistance (50%) and wedge pressure (9 mmHg), a 15-20 msec increase in P-R interval and a 60% fall in Che activity. This dose also appeared to slow repolarization of the ventricle as Q-T interval was lengthened by 17%. The 2mg/kg dose generally produced effects which were intermediate between those of the high and low doses. Mucous production and defecation were especially notable in the high dose. **Conclusions: Pyridostigmine in doses of 0.5 to 5 mg/kg i.v. in the dog produced a dose-related inhibition of plasma cholinesterase activity. Additional changes were: heart rate was reduced; cardiac contractility (dp/dt) was increased; stroke-volume was raised; cardiac output was thus only variably affected; airways resistance was markedly increased.**

INTRODUCTION

Pyridostigmine bromide is a reversible inhibitor of acetylcholinesterase. This agent increases plasma and tissue half-life and the biological effects of acetylcholine. The main therapeutic use of this agent is for management of myasthenia gravis, a disease manifested by skeletal muscle weakness resulting from reduced density of acetylcholine receptors at the neuromuscular junction.

A recent report has given information about the cardiopulmonary actions of pyridostigmine Br in conscious dogs (Enrlich et al, 1985). At a dose of 1 mg/kg (i.m.) pyridostigmine increased respiratory rate and minute volume as well as airways resistance, in resting dogs. Heart rate, cardiac output, and blood pressure were not altered. In exercising dogs, respiratory rate increased, but minute volume and airway resistance were unchanged. Heart rate was lowered, but cardiac output was not changed. Pulmonary artery pressure was elevated in both resting and exercising animals. Fifteen minutes after injection (1 mg/kg, i.m.), a plasma cholinesterase level of 40% of control was reached.

Our studies determine and correlate the cardiovascular and pulmonary actions of graded intravenous doses of pyridostigmine Br in the anesthetized dog. The range of doses studied was from that producing 1) a small but definite effect upon cardiovascular and/or pulmonary function, to a dose which produced 2) severe changes in cardiopulmonary function, just short of death. A dose producing effects intermediate to these extremes was also determined. In addition, these studies provide a correlation of blood cholinesterase activity with cardiovascular and pulmonary function before, during and after administration of pyridostigmine. The formulation of this drug which was utilized was Mestinon® sterile solution (5 mg/ml).

Our Preliminary Studies: In range finding studies in 12 anesthetized mongrel dog preparations, we found that a total dose of 5 mg/kg (i.v.) of pyridostigmine (Mestinon®) appeared to be the maximum tolerated. Higher doses of 6 and 7 mg/kg produced death by apparent respiratory paralysis in 2 of 3 dogs tested. A dose of 0.5 mg/kg was the lowest dose which consistently produced notable changes in cardiovascular and pulmonary function. A dose of 2 mg/kg produced intermediate effects.

Initial Studies

In May, 1985, we began experimental work according to the OUTLINE OF STUDIES below. Ten experiments were completed using mongrel dogs before our research was halted in June, 1985, due to a new Department of Army policy which forbade the use of pound dogs in sponsored research. Results of these studies are given in Appendix F. Studies were reinitiated in August, 1985 with pure-bred beagle dogs.

OBJECTIVES

The purpose of these experiments was to determine the effects of varying degrees of inhibition of blood cholinesterase upon cardiorespiratory variables produced by the range of doses of pyridostigmine; the lowest dose at one end, produced only minor cardiopulmonary effects and, the largest dose, at the other end, caused severe changes just short of death. We have attempted to correlate functionally graded changes in cardiovascular and pulmonary performance in response to pyridostigmine.

METHODS

I. Drug Preparation and Delivery

Pyridostigmine Br (mw = 261.14), 1-methyl-3-hydroxypyridinium bromide dimethylcarbamate, was purchased from Roche Laboratories as Mestinon® [Lot #0102 for injection, 5 mg/ml, 2 ml/ampul]. Samples of this lot were analyzed for purity and content by SRI International, Menlo Park, California. The report of this organization revealed that the injectable solution met USP specifications for content (5.00 mg, s=0.05 mg pyridostigmine), pH (5.19) and volume (2.26 ml) per ampule (Petesch et al, 1985). Lot #0101 was used in experiments with mongrel dogs. Analysis of this lot indicates that it also met USP requirements. This drug was diluted daily for each experiment with normal saline to an appropriate concentration to deliver the desired dose in a volume of 29 ml (1.91 ml/min x 15 min). Aliquots of the commercial vial contents were accurately weighed at 21°C to measure our addition of proper amounts of test drug to the infusate.

II. Animals

Beagle dogs of either sex, 9 to 12 months old, weighing between 6.7 and 14.2 kg were purchased from Ridglan Animal Care Systems (Mt. Horeb, WI) for these studies. The dogs were certified to be in excellent health and filaria free and were examined by a University of Tennessee veterinarian before use. Only dogs with normal ECG profiles were used. Food was withheld for twenty-four hours prior to the experiment. Dogs were anesthetized with pentobarbital Na, 30 mg/kg, intravenously and maintained with supplemental injections of 1.0 mg/kg as necessary to maintain stable anesthesia level. Corneal and plantar reflexes, response to pain, and respiratory rate (16-20 breaths/min) were used in "titrating" the dog to the desired level of anesthesia. Body temperature was monitored through the Swan-Ganz catheter and maintained by

heat lamps between 37-38°C. Upon completion of the experiment we euthanized the dog by injecting 10 ml of saturated KCl solution i.v. and monitored to cardiac asystole and respiratory apnea.

III. Cardiovascular Measurements

Cardiovascular variables were measured and/or computed using a Grass Model 7B polygraph and a Buxco Cardiovascular Analyzer and were recorded on both the polygraph and a Texas Instruments Model 675 data terminal.

N.B. See Appendix G for details of instrument calibration procedures.

All catheters for pressure measurements were filled initially with heparinized saline (80 units/ml) and flushed with heparinized saline as needed to maintain patency. A femoral artery was catheterized with polyethylene tubing (PE 260) advanced to the thoracic aorta and connected to a Statham P23AC pressure transducer for measurement of arterial blood pressure. The left carotid artery was cannulated with a Millar® Mikro-tip® catheter pressure transducer (Model PC-350, size 5F) for high fidelity measurement of left ventricular pressure (LVP). The rising slope of the LVP was differentiated to give dP/dt, an estimate of myocardial contractility. The left ventricular pressure signal was also used to record left ventricular end diastolic pressure. Heart rate was recorded via a Grass cardiotachometer triggered by the R wave of the Lead II ECG. Short strips of standard leads were recorded every 10 minutes at 25 mm/sec and Lead II rhythm strip was recorded every 10 minutes.

One femoral vein was catheterized to the level of the heart for drawing venous blood samples (approx. 4 ml) and the other femoral vein was cannulated for drug infusion. Arterial blood samples (approx. 5 ml) were drawn from the

aortic pressure line at selected time points. Samples were taken in glass syringes rinsed with heparinized saline following evacuation of the static volume in the cannulae. Syringes containing blood were immediately capped with tight-fitting rubber nipples and put in ice until analysis. The first samples drawn were designated as -15 minutes. At time 0, just prior to onset of the infusion of drug or vehicle, another set of blood samples were taken. Additional sets of blood samples were taken at +7.5, +15, +25, +45, +85, and +115 minutes. We drew one ml of arterial blood and transferred the blood to a glass test tube rinsed with heparinized saline, and lysed the cells by freezing. The samples were then thawed and used for analysis of cholinesterase activity. (See section V, Blood Analysis). Microhematocrit was assessed using venous blood. Withdrawal of blood was performed by a person other than the operator of the blood gas analyzer.

Cardiac and Pulmonary Vascular Function

For the measurement of cardiac output an Instrumentation Laboratories thermal dilution cardiac output computer (see appendix for theory of operation) was used. Cardiac output, pulmonary artery and wedge pressure were determined from this same catheter. Outputs were obtained in duplicate at given time points by injection of 3 ml of saline at zero degrees C and a subsequent computer integration of the temperature change vs time curve as detected by the thermistor. The two determinations for each point were taken at the same place in the respiratory cycle and averaged. A Swan-Ganz type catheter (Swan et al., 1970) with an extra injection port and a temperature sensor was connected to a Statham P23Gb pressure transducer (ultra low volume displacement) and was inserted into the right jugular vein. The balloon at the catheter tip was inflated and the catheter "floated" through the right

atrium and the right ventricle into the pulmonary artery. The distinctive changes in pressure tracings identify these locations. The balloon tip was advanced into the pulmonary artery until an abrupt drop in pressure and cessation of pulsation indicate that the balloon had occluded a secondary branch of the pulmonary artery. The balloon was then deflated (restoring the pressure pulsations) and the catheter advanced a few more mm, maintaining the normal pulmonary artery pressure pulsations. The catheter was then securely tied in place. Upon re- inflation of the balloon tip (for 15 sec), pulmonary wedge pressure (an indication of left atrial pressure) was recorded at desired time points. With the balloon deflated, pulmonary artery pressure was continuously recorded.

Pulmonary Vascular Resistance (PVR) was calculated according to the formula: PVR = (PAP - LVEDP)/CO, where PAP = pulmonary artery pressure, mm Hg; LVEDP = left ventricular end diastolic pressure, mm Hg; CO = cardiac output or pulmonary artery blood flow, L/min. Interpretation of the drug's action took into account possible flow-induced changes in the pulmonary vasculature.

IV. Pulmonary Ventilatory Measurements

N.B. See Appendix G for instrument calibration procedures and details of pulmonary ventilatory measurements.

We estimated the pulmonary function of the dogs breathing room air unassisted via an endotracheal tube with a side arm that was connected directly to a mesh screen Fleish pneumotachograph. We measured the pressure difference across the pneumotochograph screen with a Validyne® differential pressure transducer, Model MP45-24. The resultant signal, when calibrated against a known air flow, corresponds to the tidal airflow rate and, in turn, when inte-

grated, yields tidal volume. An esophageal tube (Porter ID 6.5) was inserted into the esophagus for the estimation of intrapleural pressure. The pressure difference between the airway and esophagus, or transpulmonary pressure, was measured by a second Validyne® differential pressure transducer, Model MP45-14. Dynamic airway resistance ($\Delta P/\Delta F$) and dynamic airway compliance ($\Delta V/\Delta P$) were computed using a Buxco Electronics Pulmonary Mechanics Computer Model 6. Airway resistance and compliance and tidal volume were recorded as analog signals with a Grass Model 7b polygraph and as digital values with a Texas Instruments Model 765 data terminal.

V. Blood Analyses

All samples were analyzed within 10 minutes for O_2 content by Cheryl Reick using the Corning Model 165/2 Blood Gas Analyzer . Each sample (4 ml each of arterial and venous blood) was analyzed in triplicate. Calibrations were made with standard gas mixtures.

Assay of whole blood cholinesterase was performed as described by Ellman et al. (1961) and Siakotos et al. (1969). We froze the blood samples 7-14 days prior to cholinesterase determinations. Tests in our laboratory have shown that there is no difference in results obtained from fresh blood or blood frozen for two weeks (Appendix H). Briefly, this method for assessing total whole blood cholinesterase activity involves the 20 min incubation of 10ml of blood within 300 µl of a buffer-salt-detergent solution containing [acetyl-^{14}C]-choline Iodide (New England Nuclear 4.4 mCi/mmol) and cold acetylcholine at a concentration of 10^{-3}M ($6X10^6$ cpm/ml). The reaction was stopped by addition of a suspension of Amberlite IRP-69 resin in dioxane. This resin binds unhydrolyzed (intact) acetylcholine. The resultant mixture

was centrifuged (900 g for 1 min) and an aliquot of the supernatant solution containing free ^{14}C-acetate was measured by a liquid scintillation counter. Non-enzymatically (non-specifically) produced free ^{14}C-acetate was determined by the regular assay, but by use of reaction mixture containing water rather than blood.

VI. Other Observations

Because this particular drug often causes increased mucous secretion within 5-10 mins of the start of infusion, the volume of mucous produced and released from mouth and nose was estimated by the use of a funnel and graduated cylinder under the dog's snout. Following euthanasia with KCl, the lungs were inspected grossly for abnormalities and a lung lobe was tied off and placed in water for an estimate of density. The heart and lungs were inspected for presence of filaria.

Because pilot studies showed that this drug induces defecation, frequency of defecation was noted.

VII. Data Presentation

All measurements called for in this protocol are presented in tabular form for each experiment using absolute values for each variable. Summary graphs have been constructed to show mean-percentage change from baseline (or control) which are designated 100%. Several variables are plotted as mean absolute values. Mean blood gases and hematocrit are given in tabular form. Variability for each measurement is given as mean ± 1 S.E.M. Standard error of the mean (S.E.M.) is estimated as standard deviation (σ) ÷ $\sqrt{N-1}$(Spence *et al.* 1968). For purposes of correlation of events, several related variables are plotted on a single graph. In addition, samples of actual tracings are used to illustrate typical ECG responses to various doses. Values were determined for most variables at 10 min intervals. Because spurious and

unrepresentative values may occur for any cardiac beat or respiratory cycle, a series of values for each variable for a timepoint were inspected and the representative value was chosen for that epoch.

OUTLINE OF STUDIES

We used the following protocol and experimental scheme: Approximately 60 minutes were required following induction of anesthesia to perform the necessary surgery, cannulation procedures, and to establish calibrations. This was followed by a stabilization period of 20 to 30 minutes to insure that all variables were stabilized, and this, in turn, was followed by a control period of 15 minutes during which data were recorded. The drug infusion was then begun and continued for 15 minutes. There was a post-infusion period of 120 minutes for observation of recovery.

I. **Observations - 15 minute control period**

- A. Cardiovascular Measures
  1. arterial blood pressure - continuous
  2. left ventricular pressure - continuous
     - a. dP/dt - continuous
     - b. left ventricular end diastolic pressure - continuous
  3. electrocardiogram - Lead II, strips recorded at 25 and 100 mm/sec for analysis (-15 and 0)
  4. heart rate - continuous; by cardiotachometer
  5. pulmonary vascular
     - a. pulmonary artery pressure - continuous
     - b. pulmonary wedge pressure - at 15 minute interval (-15 and 0)
     - c. cardiac output - at 15 minute interval (-15 and 0)

d. pulmonary vascular resistance - calculated for the 15 minute interval (-15 and 0)

B. Pulmonary Ventilatory Measures

1. Airways differential pressures - continuous

a. air flow - signal integrated by preprogrammed Buxco computer

b. transpulmonary pressure (bronchial minus esophageal) - signals utilized by preprogrammed computer

2. Airway integrated measures - tidal volume and minute volume - continuous

3. Airways computer measures

a. compliance - continuous = $\Delta V/\Delta P$

b. resistance - continuous = $\Delta P/\Delta F$

4. Respiratory Rate - continuous

C. Hematological Measures (at -15 and 0)

1. Blood P_{O_2} - arterial and venous (in triplicate)

2. Microhematocrit - venous (in duplicate)

3. Cholinesterase activity (as nmoles Acetylcholine hydrolyzed/ml whole blood/hr) - arterial (in triplicate)

II. Drug Infusion for 15 minutes - Observations as described in I: A, B, and C (drug infusion time = 0 - +15 minutes)

A. Measures A. and B. from section I, plotted at +7.5 and +15 minutes with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, and 4 from section I-C. at +7.5 and +15 minutes; measure 5 from section I-C. at +15 min

III. Observation period - 120 minutes post-drug

A. Measures A. and B. from section I., plotted at 10 minute intervals beginning at +25 min and continuing through +135 min - with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, 4, and 5 from section I-C. taken at +25, +45, +85, and +115 min

EXPERIMENTAL GROUPS

			DOSE	PUREBRED BEAGLES (NUMBER)
1.	Pyridostigmine Br	0.5 mg/kg	(1.9 µmoles/kg)	6 dogs
2.	Pyridostigmine Br	2 mg/kg	(7.7 µmoles/kg)	6 dogs
3.	Pyridostigmine Br	5 mg/kg	(19.1 µmoles/kg)	6 dogs
4.	Vehicle Control Solution	Normal saline		6 dogs

All doses were given in a total volume of 29 ml given at a volume-rate of 1.91 ml/min for 15 mins. The cannula was loaded with drug prior to onset of the infusion to insure accurate drug delivery. The volume of the Mestinon® solution in the vial was measured gravimetrically at 21°C to establish the desired dose concentration and saline was added q.s. to 29 ml for the set infusion rate.

Nota Bene

The conduct of these studies comply with the GOOD LABORATORY PRACTICES (GLP) regulations as published in the Federal Register, Volume 43 (247), 22 December 1978, Part II, pp 59,986-60,020 and all subsequent addenda.

RESULTS

A summary of all baseline (time zero) data appears as absolute values in Table 1. Data on range of baseline values, range of responses and time of extreme values are given in table 4. The raw experimental data for each dog are given in tables of Appendix E.

RESPIRATORY FUNCTION

TIDAL VOLUME (TV)

Baseline values for tidal volumes for the four experimental groups at time zero ranged from 142±13 to 395±105 ml/breath (Table 1). The range of baseline values was large in the control and low dose groups (Table 4) and was, in general, inversely related to the baseline respiratory rate of each dog. These data serve to point out the considerable variability for this measurement. Large variations in the response were noted in the low dose group from +35 to +135 min.

The low dose may have produced a slight depression in TV of about 10%, with the greatest effect being noted 10 min following the infusion (Fig. 1). The higher doses produced a more marked depression during drug infusion. This reduction was of similar magnitude (25-30%) for both the mid and high doses; the effects persisted through +55 minutes of the observation period. The time to peak response was dose-related, occurring earliest for the high dose and latest for the low dose (Fig. 1).

RESPIRATORY RATE

Baseline values for respiratory rate varied from 8.9±2.5 and 14.4±6.6 breaths/min in the various groups (Table 1). The greatest

variability in baseline values was in the low dose group (Table 4). One dog (Table E-10) exhibited an unusually high baseline rate (44/min), which did not appear to be a function of anesthesia. This dog had a low tidal volume.

The low dose did not alter respiratory rate (Fig. 2). Both the middle and high doses produced approximately a 100% elevation in rate. There was considerable variability in responses within the mid dose group, particularly at +7.5, 15 and 55 min. This elevation in rate persisted and waned slowly over the experimental period. One dog (Table E-16) exhibited high rates at these points.

MINUTE VOLUME

Minute volume is the product of average tidal volume and respiratory rate. Mean baseline values ranged from 1.3±0.2 to 2.9±0.5 l/min for the groups (Table 1). This variable was not affected by the low dose (Fig. 3). The mid and high doses produced similar elevations in minute volume of 35% to 50%. Wide fluctuations in minute volume over time were particularly evident for the middle dose due primarily to one dog (Table E-16). Values fell slowly toward control. Variability to this calculated measure was largely due to variability in respiratory rate.

COMPLIANCE

Absolute baseline values for compliance ranged from 23.5±3.7 to 53±10.3 CU (Table 1). There was considerable variation to individual values; in general, dogs with high tidal volumes exhibited high compliance values.

Only the high dose infusion had any definite effect upon airways compliance (Fig. 4). A gradual depression of 35-40% occurred over the 45

minute period beginning with the infusion. Compliance remained depressed over the remainder of the observation period in the high dose group. A small (10%) depression of questionable significance occurred with the infusion of the low and mid-dose.

Response ranges in all groups were extensive, but particularly in the high dose group where one dog exhibited a marked rise in compliance at +65 and 75 min (Table E-22). Tidal volume was briefly elevated over these time points. Increased variation is to be expected when toxic doses are given that seriously alter physiological functions.

RESISTANCE

Baseline resistance values ranged from 2.7±0.9 to 5.5±1.5 resistance units (Table 1). Within each group, there was a considerable range of values (Table 4).

Airways resistance was elevated in a dose-related manner (Fig. 5). By far, the most prominent rise (≈1000%) was produced by the high dose after the end of infusion (+25 and 35 min). Considerable variability was noted in the magnitude of these peak responses (Figure 5, Tables E-19, 24 and Table 4); it was most prominent in the high dose group. Resistance decreased only partially throughout the experimental period as the mean values at the end of the observation period were still elevated ≈ 300% above control levels. The mid-dose produced a rise of ≈ 220% that remained throughout the observation period. A gradual elevation of approximately 200% occurred at ≈ 40-70 min with the low dose; a great deal of this rise and variability was due to an exceedingly marked rise in resistance at +45 min in one dog (see Figure 5, Tables E-11 and 4).

Values tended to drop toward control values toward the end of the observation period.

BLOOD CHEMISTRIES

Baseline arterial PO_2 ranged from 72.8±7 to 85±5 mmHg (Table 2). Values in the high dose group were surprisingly low (Tables E-19-24). Oxygen tension in arterial samples was not generally affected by pyridostigmine (Table 2). However, a small drop in PO_2 appeared to occur at +7.5 min in the 5.0 mg/kg group and mean values over the entire experimental period were lower in this group. Baseline venous PO_2 ranged from 45±4 to 52±3 mmHg. The only apparent changes in venous PO_2 was a rise (14 mmHg) which occurred at +15 min in the high dose group.

Baseline venous blood hematocrit ranged from 39±2 to 42±3 percent cells. Hematocrit appeared to rise with the infusion of pyridostigmine. This rise was most evident in the high dose group. About a 20% rise occurred at +15 min and remained elevated throughout the whole experimental period (Table 2).

CARDIOVASCULAR FUNCTION

SYSTOLIC BLOOD PRESSURE

Baseline systolic blood pressure ranged from 134±7 to 161±15 mmHg in the four groups (Table 1). The middle and high doses elevated systolic blood pressure modestly (10%) during the infusion (Fig. 6). Immediately following the infusion, pressure fell about 10% below baseline values in these two groups over the next 20 minutes, then leveled toward control values. The low dose did not alter systolic blood pressure. Values in the control group rose abruptly at +85 min and remained elevated. This rise was mainly due to a 50 mmHg rise in one dog (Table E-4); cardiac output rose over 40% from +75 to 85 min in the dog.

DIASTOLIC BLOOD PRESSURE

Baseline diastolic blood pressure ranged from 97±5 to to 118±12 mmHg for the four groups (Table 1). The high dose produced a fall in pressure of approximately 25% at 35 minutes (Fig. 7). No important drop was evident for the low and middle dose. The reduction in pressure by the high dose persisted through +75 minutes; pressure then climbed toward control values. Large variability was noted in response of the high dose; one dog exhibited a marked anomolous rise at +7.5 min of unknown etiology (Table E-19); several others had marked drops in pressure during and just after dose administration (tables E-20, 22, 23). The abrupt rise in the control group at +85 min was due to response of one dog (Table E-4), as noted above for systolic pressure.

HEART RATE

Baseline heart rate of the groups ranged form 139±14 to 157±10 bpm (Table 1). Heart rate decreased in a dose-related manner during the

PULMONARY VASCULAR RESISTANCE (PVR)

Baseline values for pulmonary vascular resistance ranged from 5.0±0.4 to 8.6±2.3 mmHg/l/min (Table 1). This variable increased during both the mid and high dose infusions (Fig. 12). The mid dose did not increase PVR until +15 minutes; variability within this group is largely due to one dog which exhibited an abrupt increase in PAP and drop in CO at +15 min (Table E-15). Values in the mid dose group returned toward control rapidly; the response to the high dose persisted until about +75 minutes. The low dose did not alter PVR during the first hour; PVP tended to paralled the rise noted in the control group.

PULMONARY WEDGE PRESSURE (PWP)

Baseline PWP ranged from 2.9±0.5 to 3.5±0.7 mmHg (Table 1). All doses produced an elevation in PWP (Fig. 13). The low and mid doses produced similar peak increases of about 3 mmHg at the end of infusion. The high dose caused an increase in pressure of approximately 6 mmHg. As with PVR, PWP in the control group tended to rise over the experimental period; however, a large contributor to this rise and the variability was the response of one dog at +85 min (Table E-4).

BLOOD CHOLINESTERASE (Che) ACTIVITY

Baseline cholinesterase activity varied from 178.4±21 to 243.4±36 nMoles of acetylcholine hydrolyzed/ml of whole blood/hr. Cholinesterase activity dropped during the infusion in a dose-related manner; the nadir appears to occur at the end of the infusion (Fig. 14). The lowest dose caused a 40% fall in activity while the highest dose produced a 60% inhibition of enzyme activity. The mid dose produced a 50% depression.

A partial rebound of activity of 10-20% occurred in all drug groups during the 10 minute period following the infusion, but values for all doses remained consistently depressed for the remaining time period. Cholinesterase activities in the mid and high dose were similarly depressed.

ELECTROCARDIOGRAPHIC EFFECTS

Pre-drug values for P-R interval varied from 80±2 to 91±4 mSec in the four experimental groups (Fig. 15, also see Tables 1 and 3 and Fig. 16). During the mid and high dose rate infusions, P-R interval increased by about 15-20 mSec. The rise was more rapidly attained for the high dose group; the peak occurred by +7.5 min. Values in these groups fell slowly after the infusion. There may have been a slight increase in P-R interval to the low dose.

Pyridostigmine at 0.5 and 2.0 mg/kg caused no ectopic beats or changes in rhythm. In one dog, the high dose of 5 mg/kg produced some short runs of ectopic beats during the infusion and these decreased and disappeared over the 30 minutes following the infusion. A second dog at 5 mg/kg had scattered ectopics before the infusion of pyridostigmine which were maintained with no obvious change in frequency during and for an additional 30 minutes after the dose and then disappeared. It is likely that this case was unrelated to the pyridistigmine dose.

The drug had no effects on the P-wave, the T-wave, or the QRS configuration (Table 3). The P-R interval was increased slightly by the 5 mg/kg dose as might be expected, although it did not approach the upper limit of normal of 0.13 sec. (Ettinger & Suter, 1970). The major alteration was an increase in the Q-T interval which appeared at the end of the

infusion and persisted throughout the observation period at the 5 mg dose. The baseline Q-T interval was on the upper edge of normal values and increased with the 5 mg dose by 28% at 15 minutes and 38% at 60 and 120 minutes. However, when the Q-T interval was corrected for heart rate the increase dropped to 0% at 15 minutes, 11% at 60 minutes, and 17% at 120 minutes. Pyridostigmine apparently has some effect to slow repolarization of the ventricle, and this action may contribute to the occasional arrhythmias seen. Only control group and high dose group electrocardiograms are compared in Table 3 as low and mid dose groups lacked consistent changes.

Figure 16 depicts representative tracings from two dogs in each group at time zero and +15 minutes.

OTHER OBSERVATIONS

Mucous production and defecation occurred in every dog at the high dose. The quantity of mucous ranged from 60 mls to 110 mls. Both defecation and mucous production usually occurred during or just following the infusion. Responses to the mid dose were somewhat varied. Two dogs in this group showed no defecation and no significant mucous production. Mild mucous production (≈20 mls) and defection occurred in another two dogs of the mid-dose group. In the two remaining experiments one dog had mild mucous and no defecation, the other had defecation and no mucous production. The low dose caused no measurable mucous production. In two instances at the low dose, defecation occured immediately following the infusion.

Lungs excised from control and high dose group dogs demonstrated similar abilities to float in containers of water. Cross-sectional cuts of these lungs did not reveal any gross differences in mucous or fluid

content, at least in middle sized bronchioles. Mucous content of larger, secondary branch bronchioles was obviously greater in the high dose as opposed to the control group.

Summary of CARDIOVASCULAR COMPOSITE DATA (See Appendix D)

Heart rate was reduced in response to pyridostigmine in a dose related fashion. It, in some ways, mirrored the rise in P-R interval caused by this drug; possibly because both these actions were due to enhancement of vagal function; the fall in diastolic pressure correlated with the fall in heart rate. Even in spite of reductions in heart rate, cardiac output was maintained or even augmented during the infusion period. Stroke volume rose in a dose-related fashion during drug infusion. This rise in cardiac output during drug infusion was associated with an increase in cardiac contractility, LV dP/dt. Rises in both cardiac contractility and output corresponded temporally to the increases in systolic and arterial pulse pressures.

In the pulmonary vascular bed, pyridostigmine infusion increased arterial pressure at all dose-rates. Vascular resistance was also elevated in a parallel fashion. It appears that a strong direct pulmonary vasoconstriction occurred, since cardiac output was maintained or even augmented during this period. Since pulmonary wedge pressure was also elevated, this suggests that an active constriction of the pulmonary veins and/or capillaries occurred.

Summary of RESPIRATORY COMPOSITE DATA (See Appendix D)

Respiratory rate was variably elevated during the mid and high dose-rate infusion, and during this period, the average tidal volume was reduced by about 25%. Minute volume, the product of respiratory rate times tidal volume, was elevated by both the mid and high dose; the greater contributor to this response and variability was rate. Minute volume and respiratory rate were

maintained at a level higher than control for most of the observation period. Airway resistance was markedly elevated by the high dose of pyridostigmine. The rise in airways resistance was mirrored by a drop in airways compliance; but these two responses are probably not causally related.

DISCUSSION

Pyridostigmine is a reversible inhibitor of choline esterase, the enzyme which destroys and stops the actions of the neurotransmitter, acetylcholine. In general, the pharmacological properties of anticholinesterase agents, like pyridostigmine, can be predicted merely by knowing those loci where choline is released physiologically by nerve impulses and the responses of corresponding effector organs. However, the many and diverse locations of cholinergic synapses increase the complexity of the response. Potentially, an anticholinesterase agent which can traverse lipid membrane barriers can produce all of the following effects: (1) stimulation of muscarinic receptor responses at autonomic effector organs; (2) stimulation, followed by depression or paralysis, of all autonomic ganglia and skeletal muscle (nicotinic actions); and 3) stimulation, with possible subsequent depression of cholinergic receptor sites (primarily muscarinic) in the CNS (Taylor, 1985).

Cholinesterase activity in blood was reduced in a dose-related fashion by pyridostigmine. Reduction in activity was greatest at the end of drug infusion. There was a partial (10-20%) recovery of enzyme activity over the 10 minute period immediately after the end of drug administration. A maintained reduced state of inhibition was then apparent for the rest of the experimental period. This partial recovery may have been due to a redistribution of pyridostigmine from blood to other body compartments.

Cardiovascular Effects

The low dose of pyridostigmine was chosen to have only minor effects and this was certainly true with regard to the cardiovascular system. Only on heart rate and perhaps briefly on diastolic blood pressure could one see a

possible effect of the 0.5 mg/kg dose. Heart rate proved to be the most sensitive and dependable indicator of the presence of pyridostigmine, showing a progressive decrease as the dose was raised. At the high dose, the duration of the bradycardia was such that the heart rate was still not back to the control value after 120 minutes. The contractility of the ventricle (dP/dt) was increased for a short time by the two higher doses; this may be partly due to an increase in and ventricular filling related to the reduction in heart rate and resistance. Resultant stretching of the heart could enhance contractions through the Starling mechanism. This proposition is certainly substantiated by the values of stroke-volume which rose in a dose-related manner during drug infusion and mirrored the dose related falls in heart rate. The simultaneous occurrence of a transitory increase in cardiac output during the infusion may also be explained in this same way. Nicotinic stimulation of sympathetic nervous ganglia can not be discounted as a means of enhancing cardiac contractility; however, the bradycardia observed during this period does not corroborate this possibility. The fall in blood pressure, which was more prominent with diastolic than systolic, is no doubt related to the cholinergic actions of pyridostigmine to slow heart rate and dilate peripheral blood vessels. The cholinergic effects were also seen in a mild increase in conduction time across the A-V node (increase in P-R interval) and a somewhat greater interference with repolarization in the ventricle. One animal developed bouts of ectopic beats for a short time following the high dose which may have been associated with the change in conduction and repolarization of the heart.

Effects of pyridostigmine on the pulmonary circulation differed from that seen in the peripheral circulation. Increases in pulmonary artery pressure and vascular resistance have been described in response to pyridostigmine (Taylor, 1985 and Ehrlich et al. 1985). The nature of this response is not clear. The increase in pulmonary wedge pressure, an estimate of left atrial pressure, was an unexpected observation since cardiac contractility and output were maintained after pyridostigmine administration. This rise in wedge pressure which also reflects pressures in the pulmonary capillaries and veins may be a consequence of constriction of these structures.

Blood PO_2 gases were generally not affected by administration of pyridostigmine. An exception may have been the increase in venous blood PO_2 noted during the high dose infusion. A possible reason for this effect could be a depression in O_2 utilization by peripheral body tissues caused by pyridostigmine. A rise in hematocrit which was particularly evident at the end of the high-dose infusion may have been due to dehydration as a result of loss of fluid through marked mucous production during this period. Another possible explanation is that contraction of the spleen through stimulation of celiac sympathetic ganglia or through reflex sympathetic stimulation pushed cell-rich blood into the circulation.

Respiratory Effects

Pyridostigmine produced prominent tachypnea at the highest doses which persisted throughout the experimental period. Central nervous stimulation within the respiratory center is possibly responsible (Taylor, 1985, Borland et al., 1985). The quaternary nitrogen in its structure makes this agent relatively lipid insoluble and less able to traverse the blood-brain barrier; however, respiratory function neural pathways are near the area postrema, a region devoid of the blood-brain barrier. The drop in tidal volume observed

at these doses is probably the passive consequence of a shorter respiratory cycle. Whether or not a paralysis of chest wall skeletal muscles was involved in the depression of tidal volume is uncertain, but unlikely, since minute volume was maintained or even augmented at the doses tested. Lack of blood gas changes confirmed that adequate ventilation was occurring.

Airways resistance was elevated by administration of pyridostigmine; peak responses were dose related. The increased airways resistance was particularly notable in the high dose group (≈1000%). The rise in resistances was probably a consequence of a buildup of acetylcholine, an effective bronchoconstrictor. Vagal acetylcholine-containing fibers are known to course to the bronchiolar smooth muscle (Sanford, 1976). Additionally, acetylcholine of cardiac origin would be expected to spill-over into the pulmonary circulation in the presence of acetylcholinesterase inhibition. An additional contributor to the rise in airways resistance is probably an increase in bronchial secretions and thus some luminal obstruction (Slonin and Hamilton, 1971). Mucous secretions in the oral and nasal cavities were certainly profuse in the high dose group. Although we saw no gross evidence for increased mucous secretion in small-size bronchioles at autopsy, we cannot exclude the possibility of enhanced mucous secretion from bronchiolar glands.

Airways compliance was definitely depressed by the high dose of pyridostigmine. Deficiency or dilution of pulmonary surfactant are prominent causes for a loss in compliance (Slonin and Hamilton, 1971). This lecithin-containing substance keeps alveoli from collapsing and allows larger increases in lung volume to occur without a rise in pressure. Dilution of surfactant with bronchiolar mucous secretions may be responsible for the fall in compliance.

Previous experiments performed in mongrel dogs given these same dose-rates of pyridostigmine revealed that responses were generally similar to those observed in beagles. Notable exceptions were that airways resistance was not raised as much in mongrels and that P-R interval was lengthened more in mongrels.

CONCLUSION

Pyridostigmine in doses of 0.5 to 5 mg/kg i.v. in the dog produced a dose-related inhibition of plasma cholinesterase activity. All of the cardiopulmonary changes can be related to the effects of Pyridostigmine directly on cholineserase activity or through effects subsequent to cholinesterase inhibition. Heart rate, which was marked reduced, and airways resistance, which was markedly elevated, appear to be the variables most affected. Other changes in cardiovascular and pulmonary function may be considered as consequences of these primary events.

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CARDIOVASCULAR AND PULMONARY EFFECTS OF INTRAVENOUS PYRIDOSTIGMINE BROMIDE INFUSION IN THE DOG; CORRELATION WITH BLOOD CHOLINESTERASE INHIBITION

FEBRUARY, 1986

These studies were conducted in accordance with current Good Laboratory Practice Regulations of the Food and Drug Administration, dated December 22, 1978, with subsequent amendments.

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APPENDIX A
LEGEND OF TERMS USED

Abbreviation	Definition
TV-ml/breath	Tidal Volume-ml/breath
Resp. rate	Respiration Rate-breaths/min
MV-L/min	Minute Volume-liters/min
C-cu	Respiratory Dynamic Compliance-compliance units
R-ru	Respiratory Dynamic Resistance-respiratory units
SBP-mmHg	Aortic Systolic Blood Pressure-mmHg
DBP-mmHg	Aortic Diastolic Blood Pressure-mmHg
ABP-mmHg	Aortic Blood Pressure
HR-beats/min	Heart Rate-beats/min
C.O.-L/min or CO	Cardiac Output-liters/min
SV-ml/beat	Stroke Volume-ml/beat
dP/dt-mmHg/sec	Acceleration of pressure, a quantitative expression for defining contractility of the heart
PWP-mmHg	Pulmonary Wedge Pressure - an estimate of left atrial pressure
PAP-mmHg	Pulmonary Artery Pressure
PVR-mmHg/l/min	Pulmonary Vascular Resistance
A-Po_2-mmHg	Arterial Blood Oxygen Tension
V-Po_2-mmHg	Venous Blood Oxygen Tension
Hct-% cells	Hematocrit-% Red Blood Cells
Ache	nmoles acetylcholine hydrolyzed/ml of whole blood/hr

Appendix B

Tables 1, 2, 3 and 4

Note: All variation bars represent S.E.M.

TABLE 1

Baseline Values Table 1 (time zero) - Beagles

	Group			
	0.5	2.0	5.0	Control
TV Ml/breath	224±64	142±13	274±62	395±105
Resp. rate (breaths/min)	14.4±6.6	9.3±1.6	8.9±2.5	10.7±3.3
M.V. (L/min)	2.2±0.4	1.3±0.2	1.9±0.3	2.9±0.5
C (CU)	44±12.3	23.5±3.7	45.1±10.8	53.0±10.3
R (RU)	3.4±0.8	2.8±0.5	5.5±1.5	2.7±0.9
SBP mm Hg	161±15	147±10	159±10	13[illegible]7
DBP mm Hg	118±12	112±9	117±9	97±5
HR beats/min	157±10	149±5	144±10	139±14
SV ml/beat	11.9±1.6	10.6±1.7	16.3±1.5	13.4±2.0
C.O. L/min	1.85±0.28	1.56±0.25	2.30±0.19	1.79±0.19
dP/dt mm Hg/sec	2395±354	2336±214	2210±278	1837±246
MPAP mm Hg	11.2±1.9	11.6±1.5	11.5±1.1	10.7±0.6
PVR mm Hg/l/min	6.0±0.7	8.6±2.3	5.0±0.4	5.8±0.6
PWP mmHg msec	2.92±0.5	3.17±0.6	3.5±0.7	3.08±0.6
P-R interval msec	86±4	80±2	84±5	91±4
Ache (nmoles acetylcholine hydrolyzed/ml of whole blood/hr)	243±36	178±21	231±32	20±31

Values given are mean ± S.E.M. (N=6, each group)

TABLE 2
Blood Chemistries - Beagles

Arterial Blood PO_2 (mm Hg)

Group	Time-min. -15	0	7.5	15	25	45	85	115
Control	75±5	84±5	80±6	81±6	84±6	87±5	74±10	89±12
0.5	75±5	75±8	75±8	84±7	80±5	81±7	85±8	84±7
2.0	70±7	85±5	83±5	75±8	85±7	94±5	90±5	91±10
5.0	73±4	73±7	53±7	66±8	62±6	65±4	75±2	72±5

Venous Blood PO_2 (mm Hg)

Control	54±5	52±3	47±5	45±5	46±5	47±3	45±5	47±7
0.5	44±5	45±5	47±3	47±6	48±5	43±6	45±6	42±5
2.0	47±5	51±4	53±5	52±6	52±5	48±5	38±3	45±5
5.0	48±5	45±4	47±5	61±8	51±5	51±2	52±3	49±3

Hematocrit % cells

Control	40±3	39±2	36±6	38±2	41±3	40±0.5	38±1	37±2
0.5	41±3	42±3	40±2	45±1	44±2	42±2	41±3	41±2
2.0	41±1	41±1	41±3	46±3	45±3	43±6	42±2	42±2
5.0	40±1	42±1	49±1	52±1	53±1	53±2	51±2	50±2

Values given are mean ± 1 S.E.M.

TABLE 3

SUMMARY OF ECG CHANGES

Item	VEHICLE (Saline) 0	15	60	120 min	PYRIDOSTIGMINE - 5mg/kg 0	15	60	120 min
Heart Rate	139	134	128	118	142	78	92	103
P-wave	normal	normal	normal	normal	normal	normal	normal	normal
††P-R interval	0.091	0.093	0.095	0.097	0.084	0.101	0.095	0.090
QRS Config	normal	normal	normal	normal	normal	normal	normal	normal
††QRS Width	0.036	0.037	0.037	0.038	0.033	0.033	0.034	0.033
††Q-T Interval	0.2	0.24	0.24	0.265	0.205	0.263	0.283	0.283
T-wave	normal	normal	normal	normal	normal	normal	normal	normal
U-wave	absent	absent	absent	absent	absent*	absent	absent	absent
Rhythm	N-S†	N-S	N-S	N-S	N-S**	N-S	N-S	

*U-wave present in 2 dogs of the pyridostigmine group at control time only

**One dog developed intermittant ectopics during and for approximately 30 minutes after infusion. A second dog had scattered ectopics before and after infusion or about 30 minutes.

†Normal sinus rhythm

††Intervals and width in msec.

TABLE 4

Range of baseline values at time zero, range of responses (percent of control) and time (min) of extreme responses.

Variable	Group	Baseline Range-Units	Range of Reponse (min)	
			Low	High
T.V. (ml)	Control	95-672	47(75)	159(135)
	0.5	68-480	49(95)	186(75)
	2.0	101-177	31(7.5)	118(15)
	5.0	106-451	52(15)	111(65)
Resp. rate	Control	3.1-24.0	48(105)	239(45)
(breath/min)	0.5	4.6-43.9	55(95)	320(85)
	2.0	5.2-15.1	46(135)	548(15)
	5.0	3.7-17.2	85(135)	297(105)
M.V. (L/min)	Control	1.7-4.6	59(75)	179(45)
	0.5	1.2-3.3	66(95)	158(85)
	2.0	0.8-1.9	61(105)	300(135)
	5.0	1.4-2.9	56(15)	300(75)
C (CU)	Control	17.7-85.6	65(125)	145(135)
	0.5	18.4-87.8	69(55)	113(105)
	2.0	13.0-36.4	67(15)	143(35)
	5.0	26.2-78.3	30(35)	190(65)
R (RU)	Control	0.5-5.4	45(125)	333(85)
	0.5	1.4-6.4	68(75)	1066(45)
	2.0	1.4-4.5	128(45)	657(55)
	5.0	1.1-8.8	24(95)	3254(35)

TABLE 4 (continued)

Variable	Group	Baseline Range	Range Response (time, min)
SBP (mmHg)	Control	121-160	81(45) - 130(85)
	0.5	115-190	71(95) - 111(15)
	2.0	120-177	68(115)- 140(115)
	5.0	130-180	60(7.5)- 133(15)
DBP (mmHg)	Control	84-115	78(45) - 115(115)
	0.5	92-149	70(95) - 109(7.5)
	2.0	91-147	61(115)- 136(135)
	5.0	88-140	50(35) - 122(135)
HR (beats/min)	Control	108-180	69(135)- 104(35)
	0.5	129-194	64(105)- 117(135)
	2.0	135-166	59(25) - 108(7.5)
	5.0	99-162	35(15) - 93(7.5)
SV (ml/beat)			
	Control	8.9-20.4	7.7(115)-30.0(125)
	0.5	7.9-17.5	5.7(135)-22.0(15)
	2.0	5.3-16.1	3.8(55) -19.9(7.5)
	5.0	11.0-19.4	11.0(0) -31.7(15)
C.O. (L/min)	Control	1.44-2.55	63(125)- 133(85)
	0.5	1.27-2.94	46(125)- 120(7.5)
	2.0	0.82-2.30	49(115)- 243(7.5)
	5.0	1.78-2.74	56(95) - 136(7.5)
LV dP/dt	Control	1119-2580	71(125)- 137(115)
(mmHg/sec)	0.5	1356-3363	52(95) - 119(25)
	2.0	1779-3078	50(65) - 142(15)
	5.0	1223-2850	66(35) - 152(105)

TABLE 4 (continued)

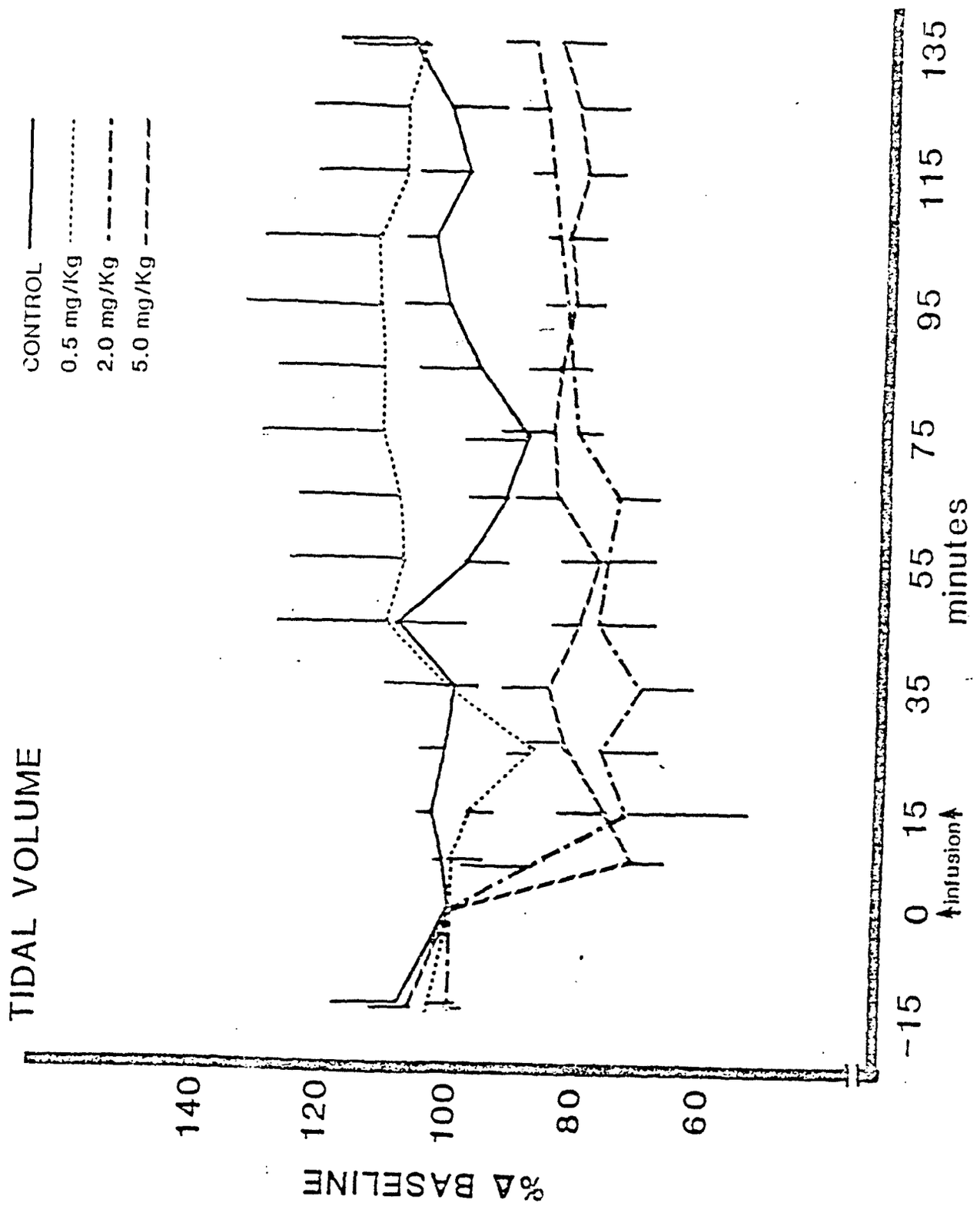
Variable	Group	Baseline Range	Range Response (time, min)
PAP (mmHg)	Control	8.7-12.1	39(75) - 161(115)
	0.5	4.9-17.2	52(95) - 157(15)
	2.0	9.5-15.9	40(135)- 178(115)
	5.0	8.2-12.7	19(75) - 220(15)
PVR (mmHg/1/min)	Control	4.39-8.40	48(75) - 166(135)
	0.5	3.86-7.77	66(75) - 209(125)
	2.0	3.32-18.20	45(55) - 234(25)
	5.0	3.69-6.02	26(75) - 210(15)
PWP (mmHg)	Control	2.0-5.5	1.0(135)- 15.0(95)
	0.5	1.5-4.0	1.5(7.5)- 12.0(15)
	2.0	1.0-3.5	1.0(75) - 11.0(15)
	5.0	1.0-5.0	1.0(15) - 13.5(125)
P-R Interval (mSec)	Control	78.5-103.5	78.5(7.5)- 113(75)
	0.5	71-95	71(7.5) - 106(135)
	2.0	73-88	68(135) - 134(7.5)
	5.0	74.5-105.5	75.5(15) - 1265(7.5)
Cholinesterase Activity (nmoles Ach hydrolyzed/ml blood/hr)	Control	181.5-304.7	67(15) - 105(15)
	0.5	152.1-374.5	43(15) - 97(25)
	2.0	100.3-283.5	37(15) - 75(25)
	5.0	87.7-272.1	26(15) - 77(115)

Appendix C

Variable Plots

Note: All variation bars represent S.E.M.

figure 1


TIDAL VOLUME
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
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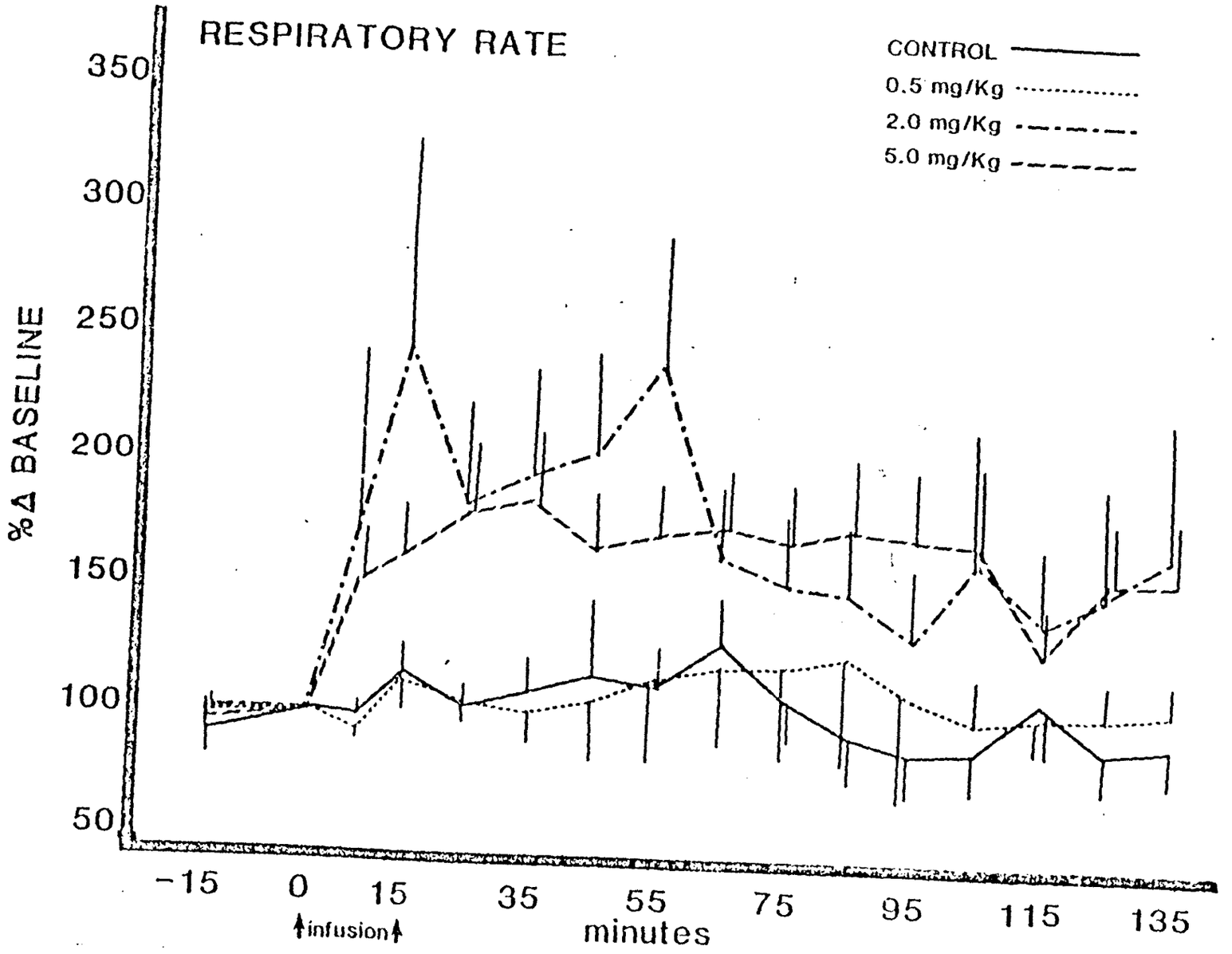
120
100
80
60
-15
0
15
35
55
75
95
115
135
minutes
Infusion

figure 2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2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
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300
250
200
150
100
50
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 3

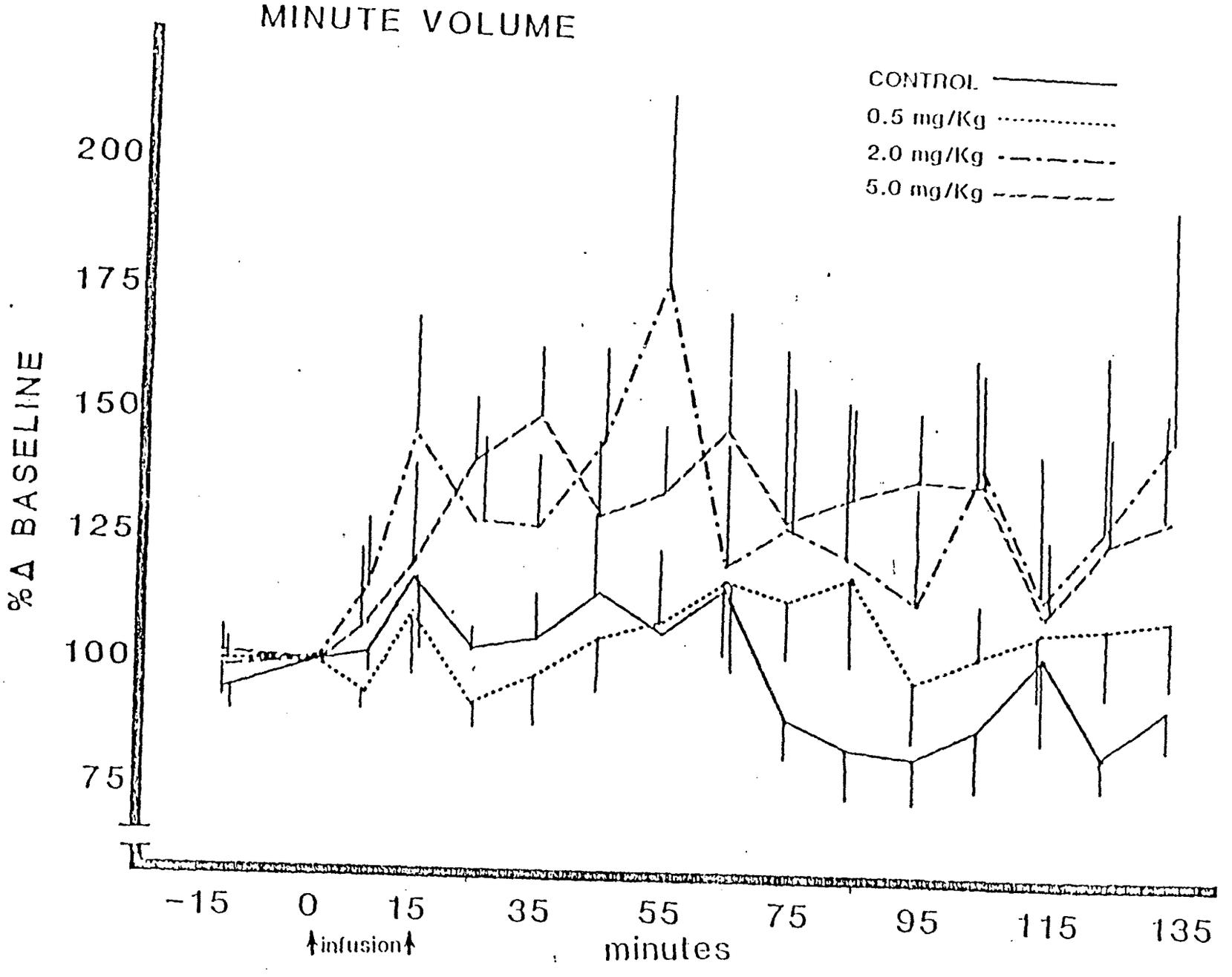

MINUTE VOLUME
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
200
175
150
125
100
75
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 4

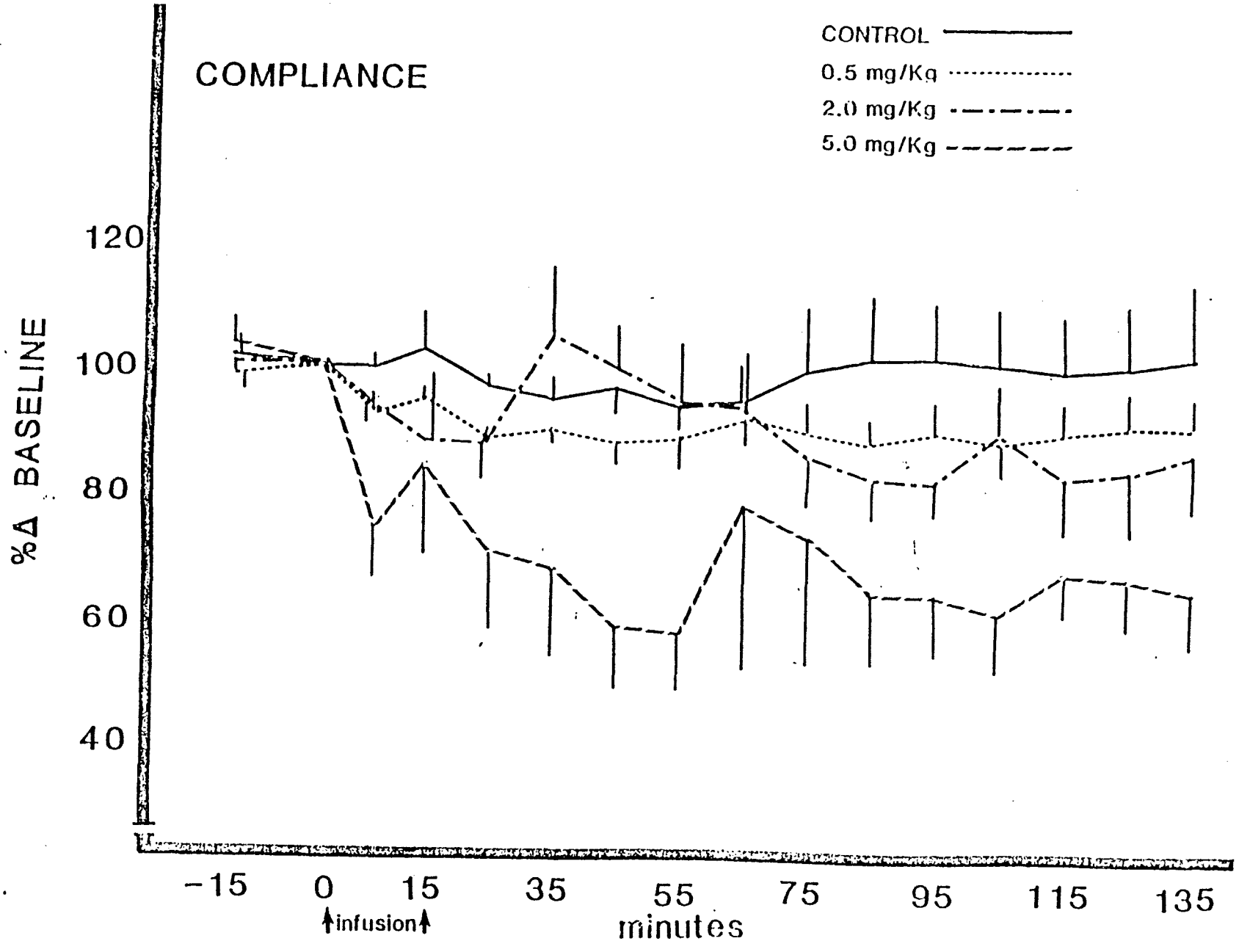

COMPLIANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
120
100
80
60
40
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 5

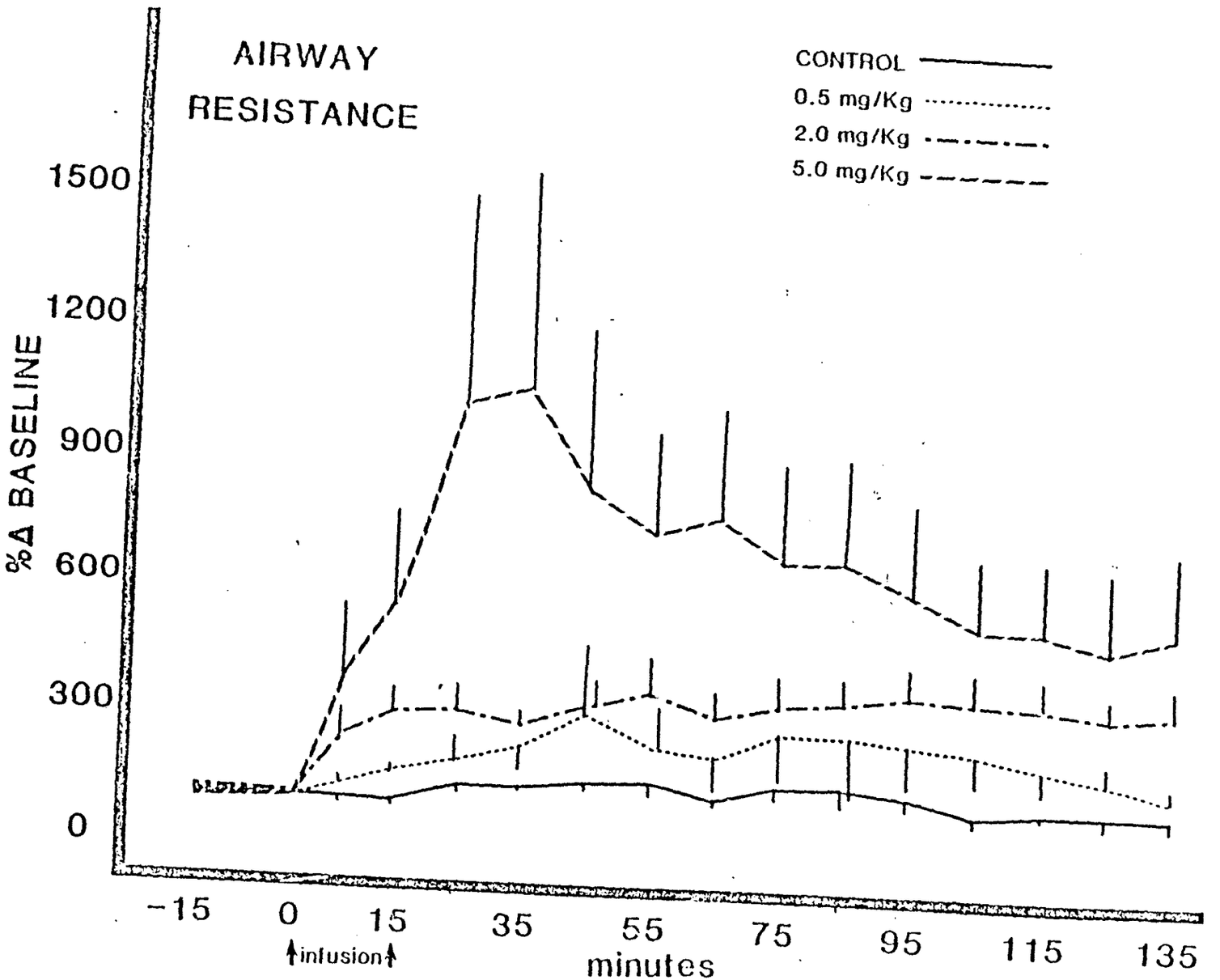

AIRWAY
RESISTANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
1500
1200
900
600
300
0
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 6

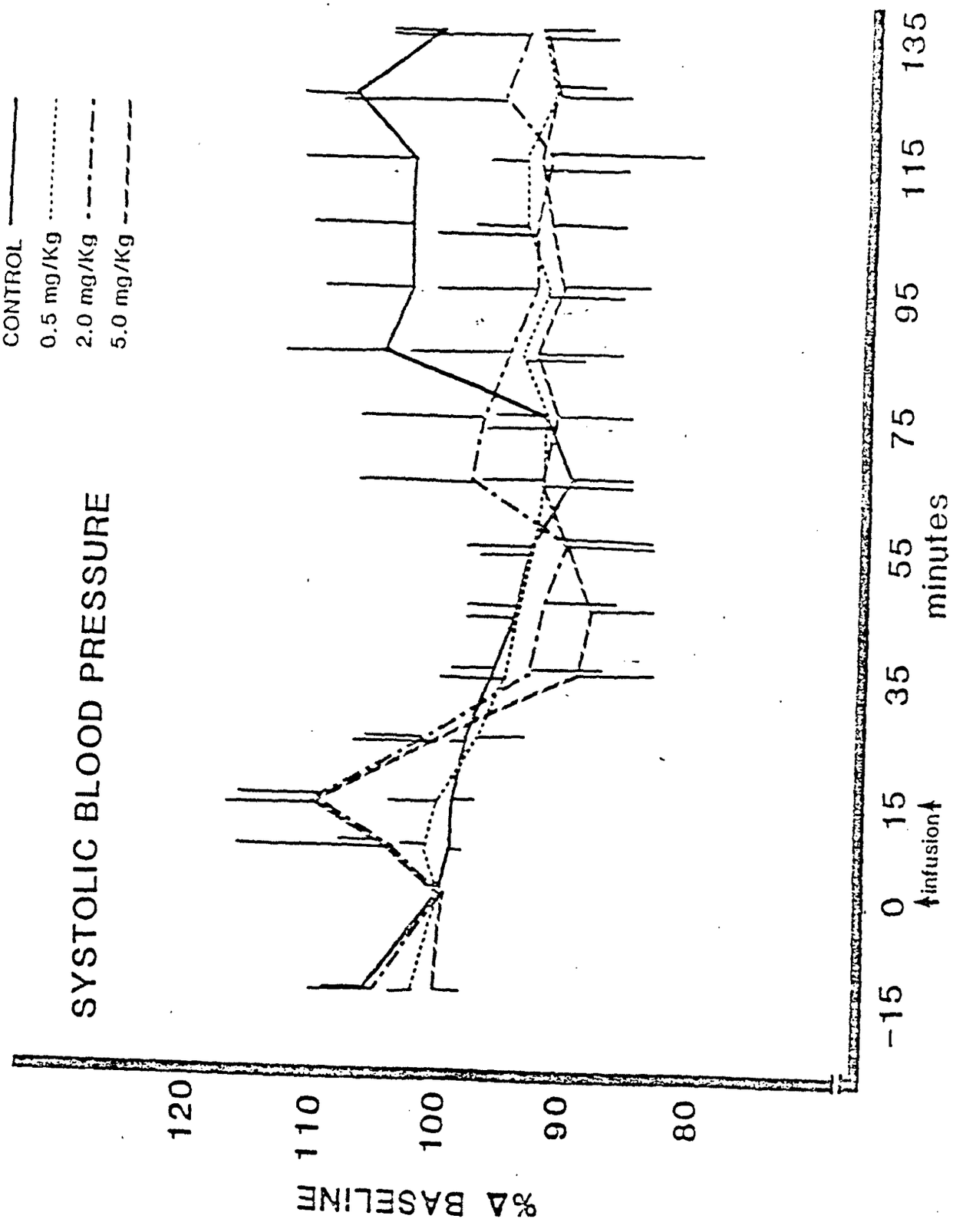

SYSTOLIC BLOOD PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
120
110
100
90
80
-15
0
15
35
55
75
95
115
135
minutes
infusion

figure 7

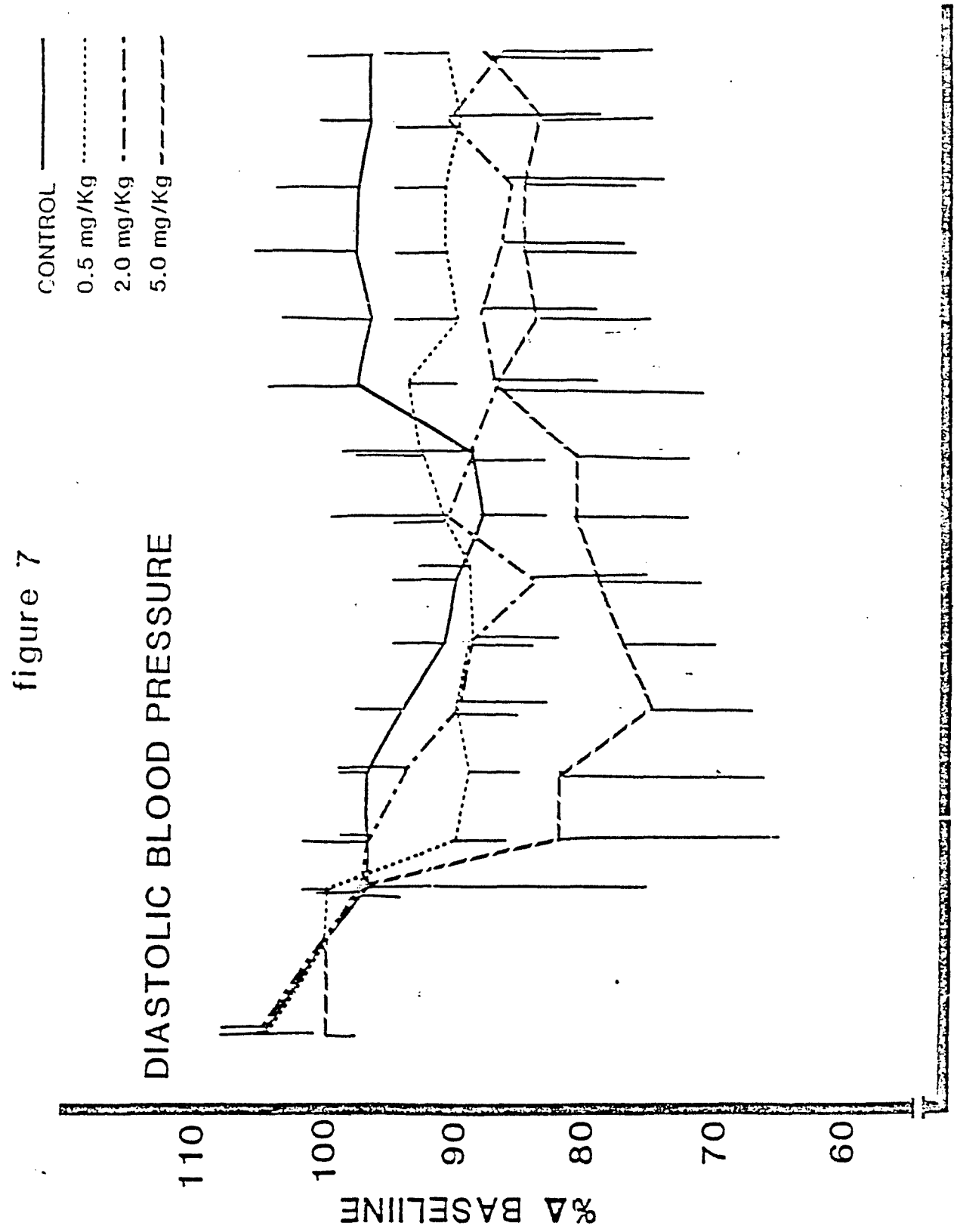

DIASTOLIC BLOOD PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
110
100
90
80
70
60
-15
0
15
35
55
75
95
115
135
minutes
infusion

figure 8

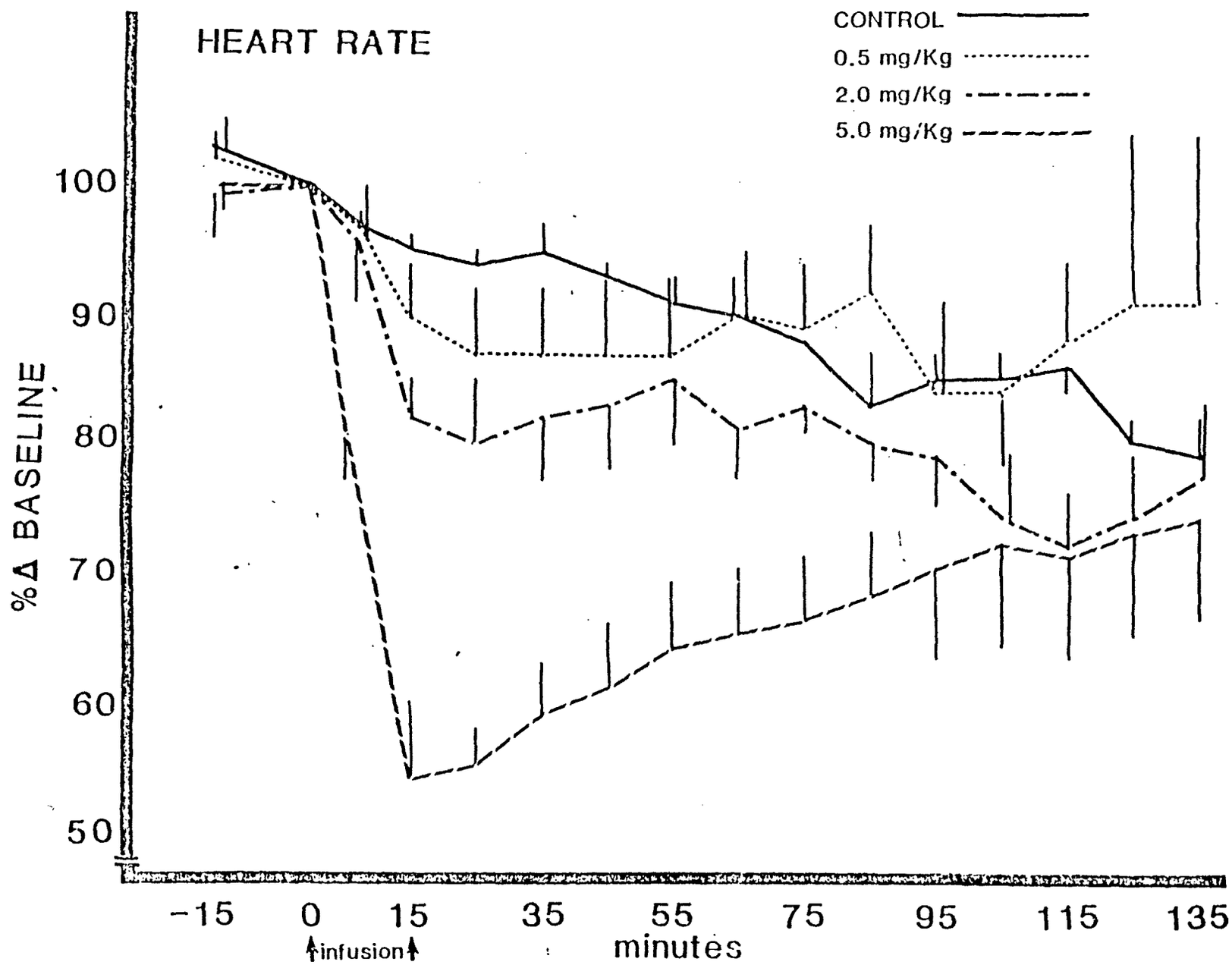

HEART RATE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
100
90
80
70
60
50
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 9

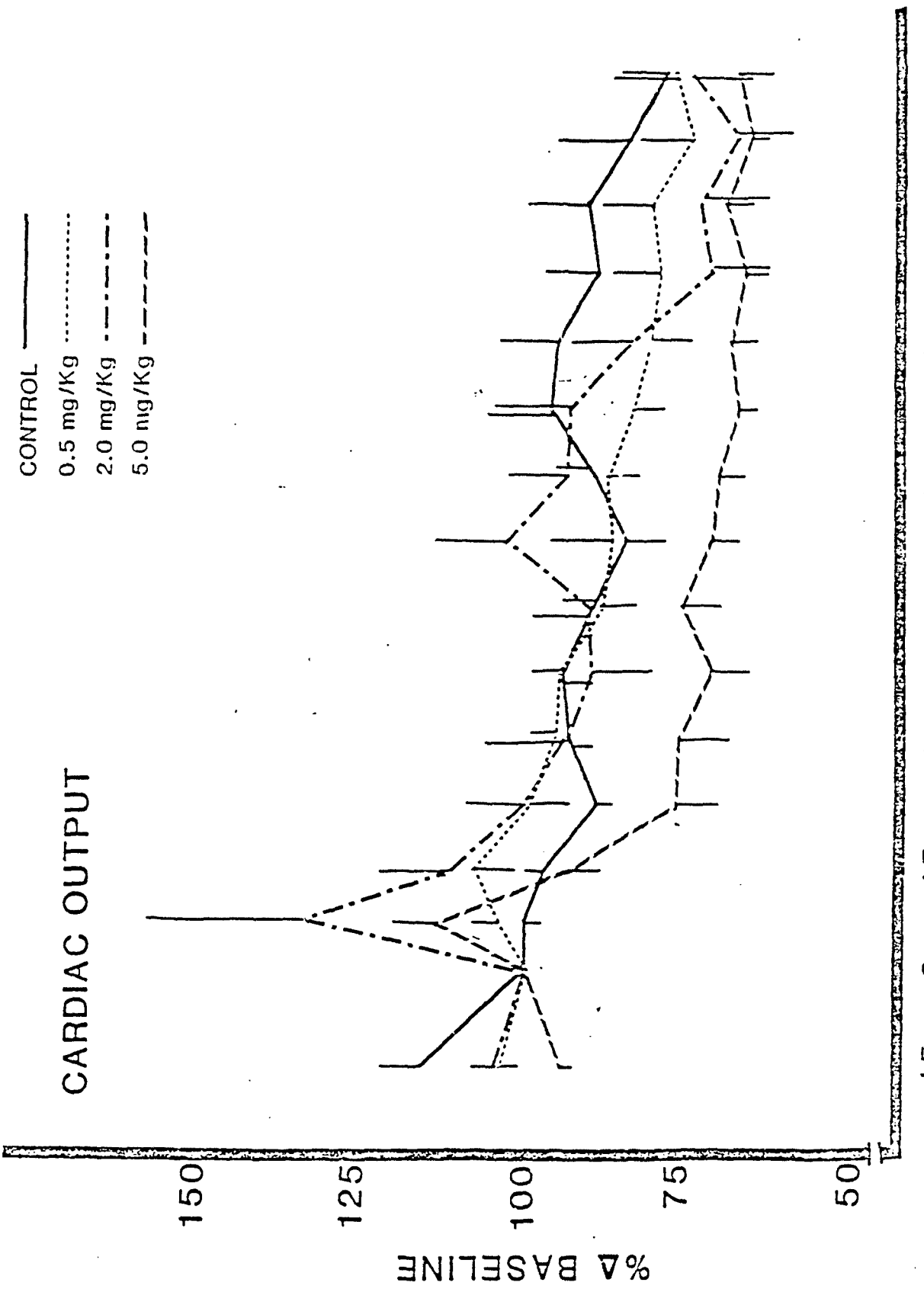

CARDIAC OUTPUT
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
150
125
100
75
50
-15
0
15
35
55
75
95
115
135
minutes
infusion

Figure 9a

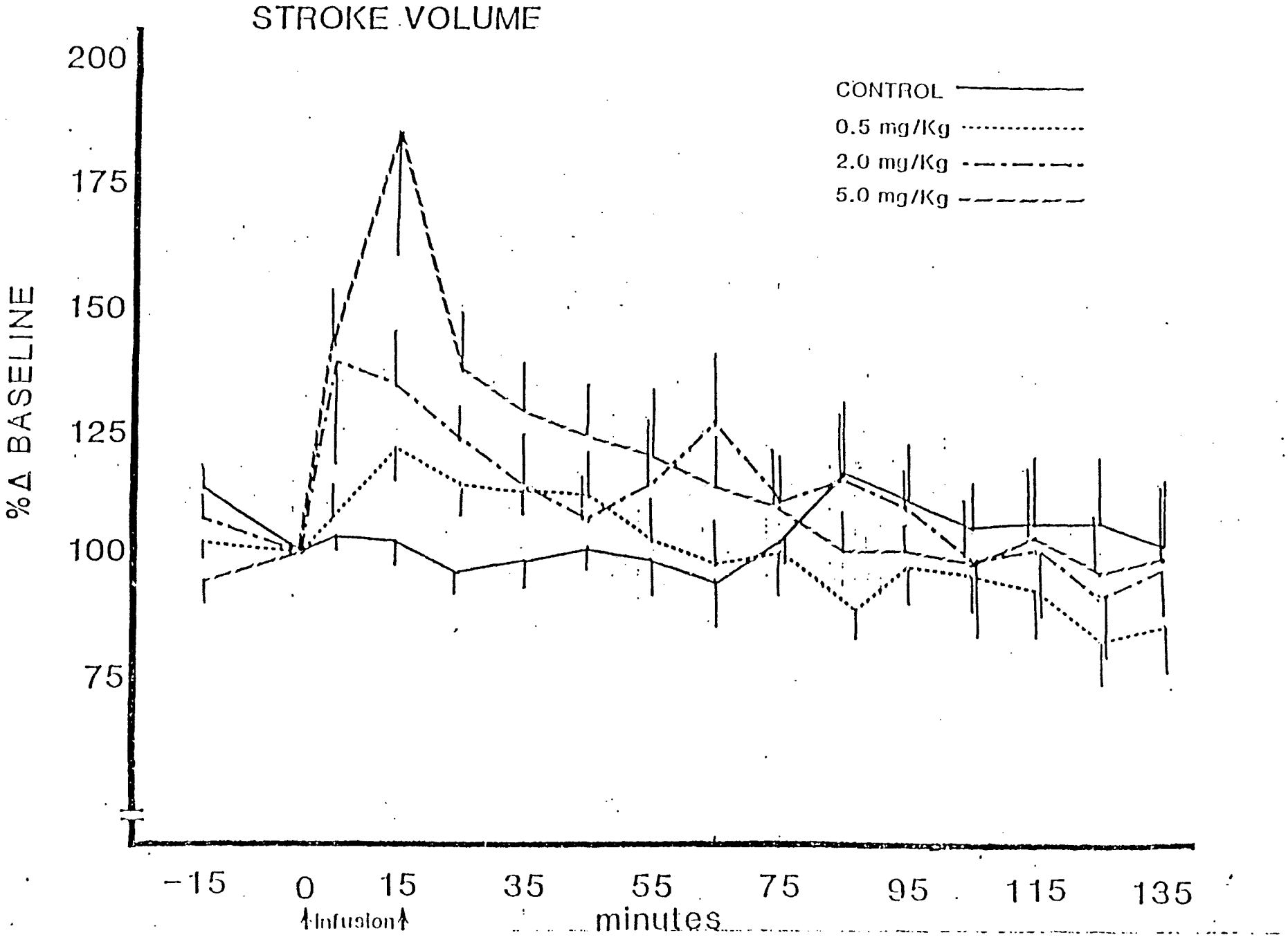

STROKE VOLUME
200
175
150
125
100
75
%Δ BASELINE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
-15
0
15
35
55
75
95
115
135
Infusion
minutes

figure 10

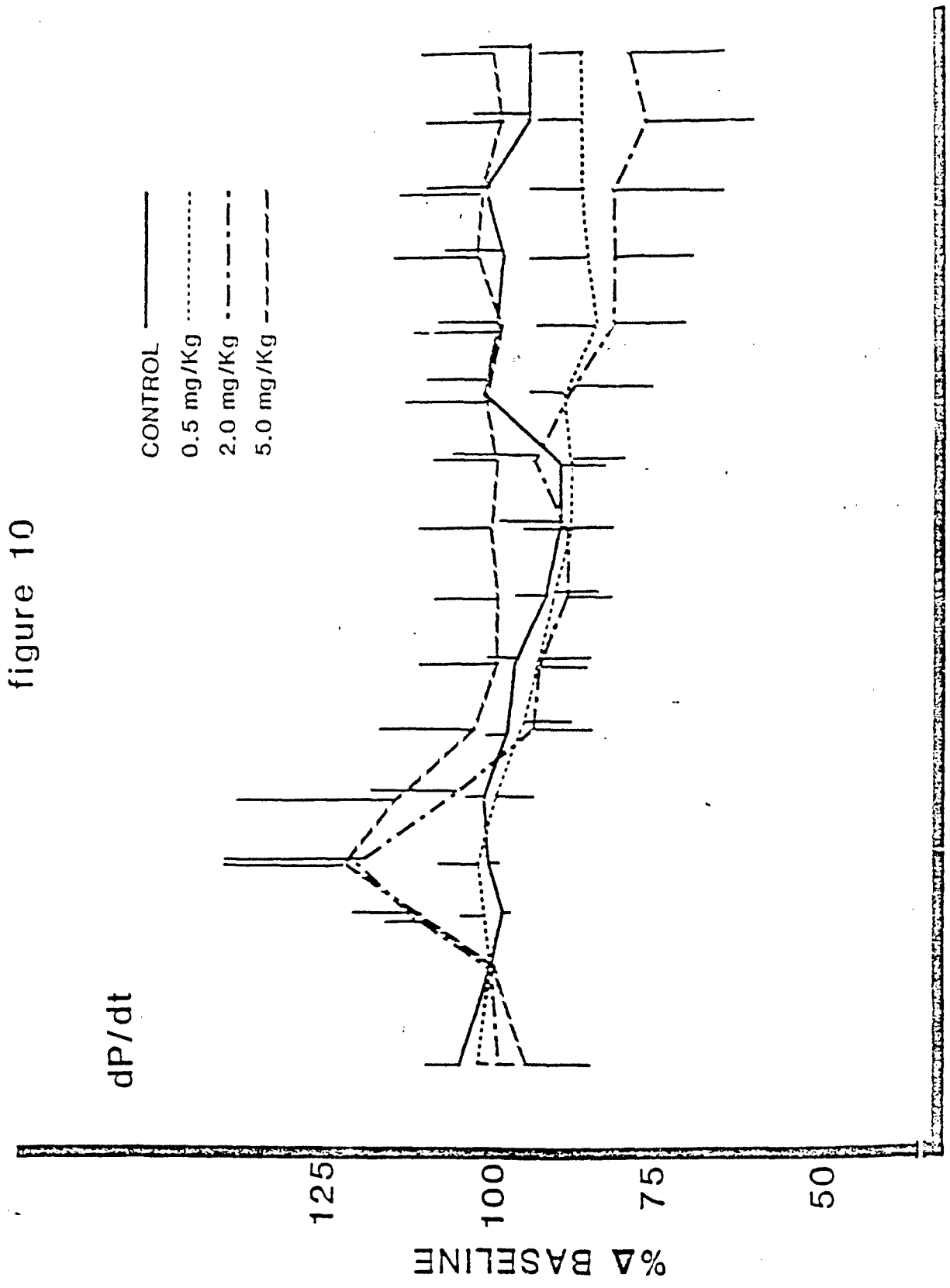

dP/dt
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
125
100
75
50
-15
0
15
35
55
75
95
115
minutes
infusion

figure 11

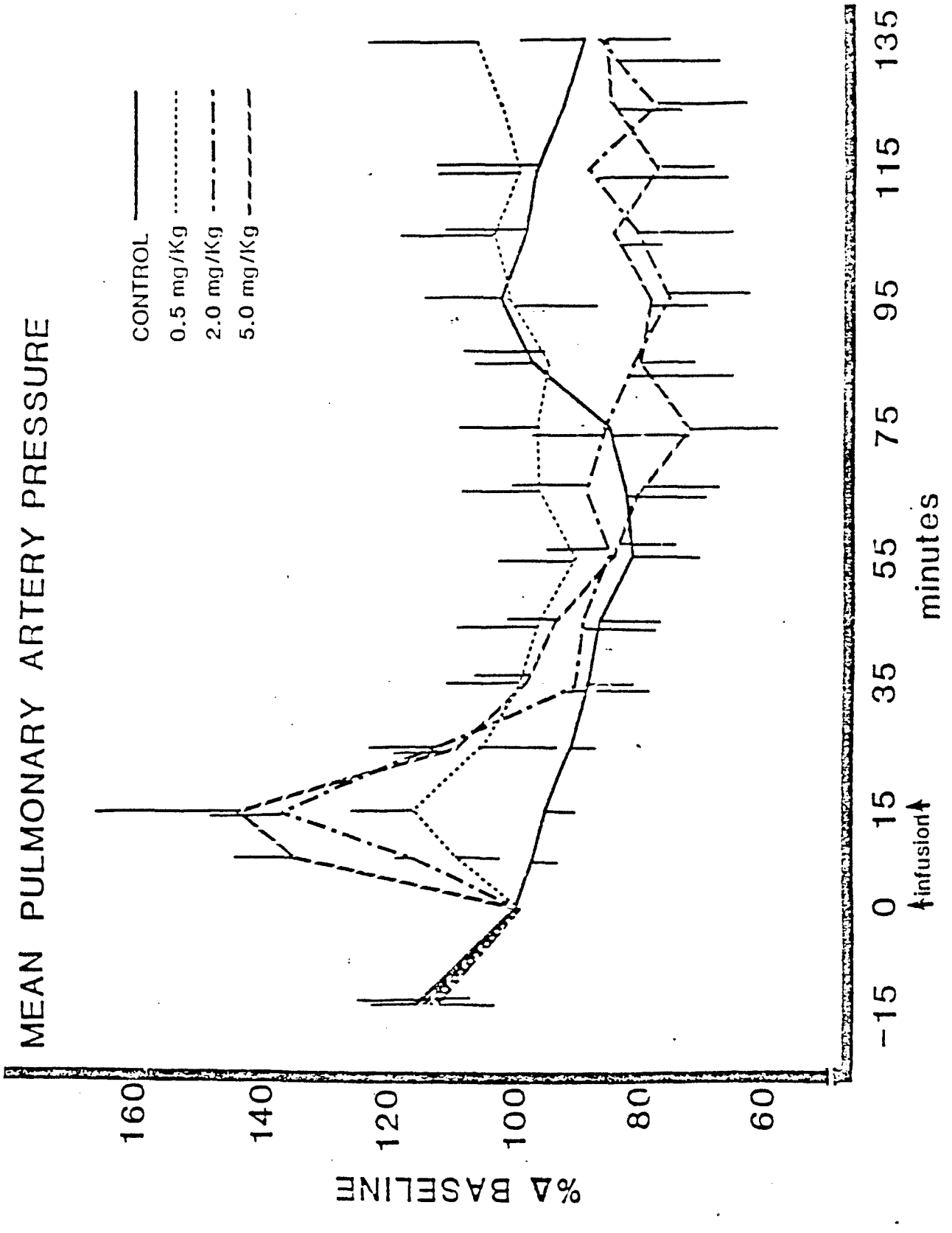

MEAN PULMONARY ARTERY PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
160
140
120
100
80
60
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 12

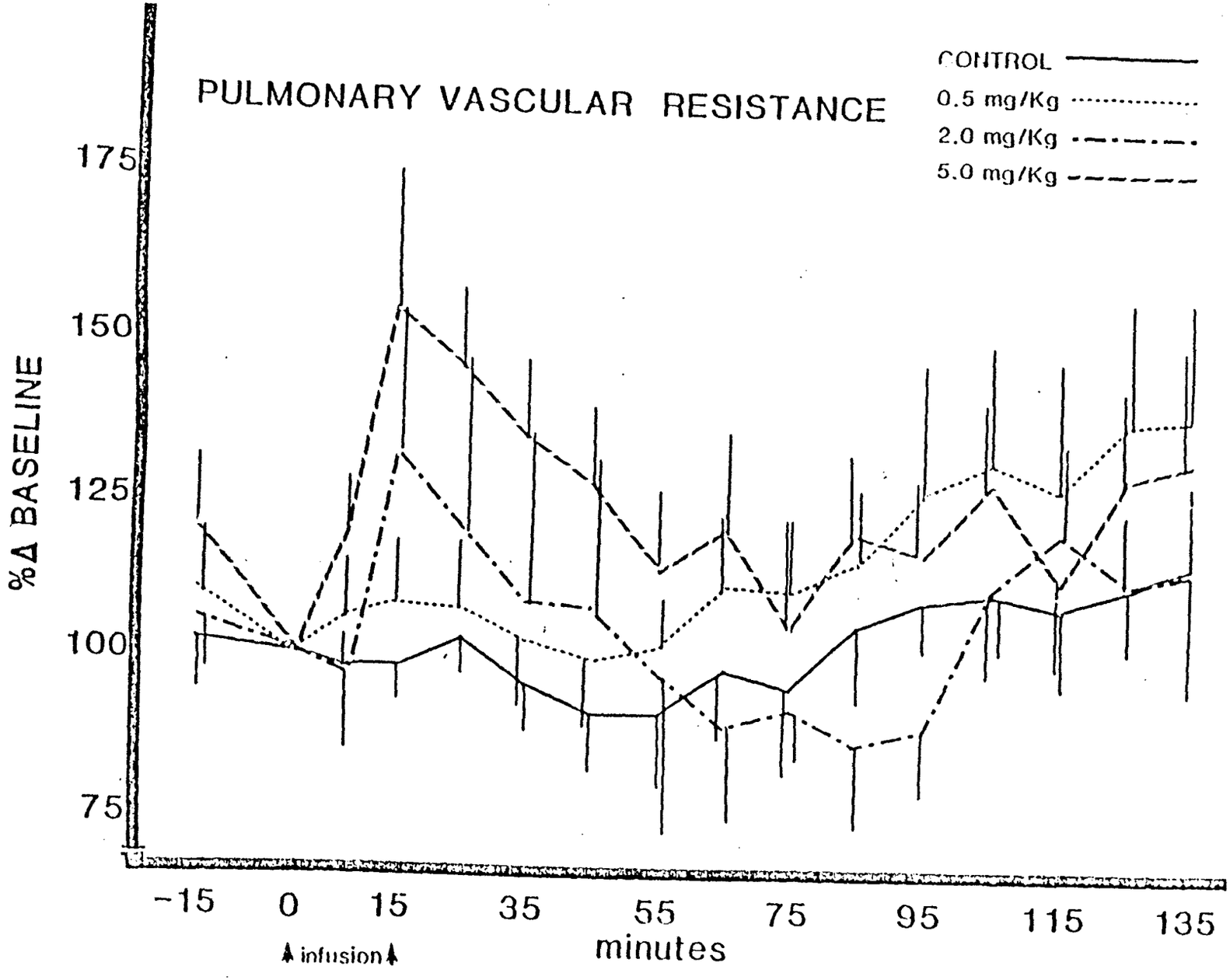

PULMONARY VASCULAR RESISTANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
175
150
125
100
75
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 13

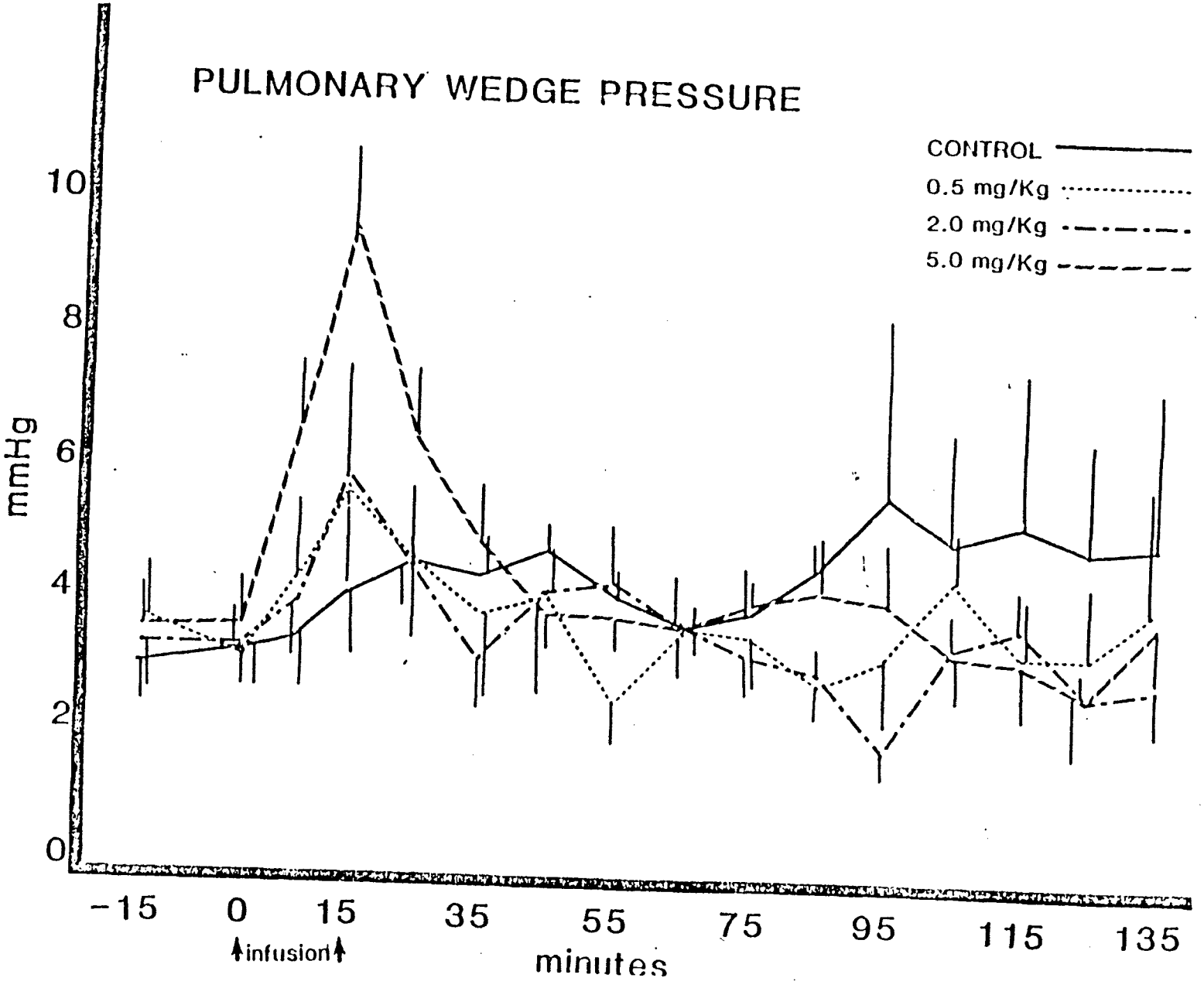

PULMONARY WEDGE PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
mmHg
10
8
6
4
2
0
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 14

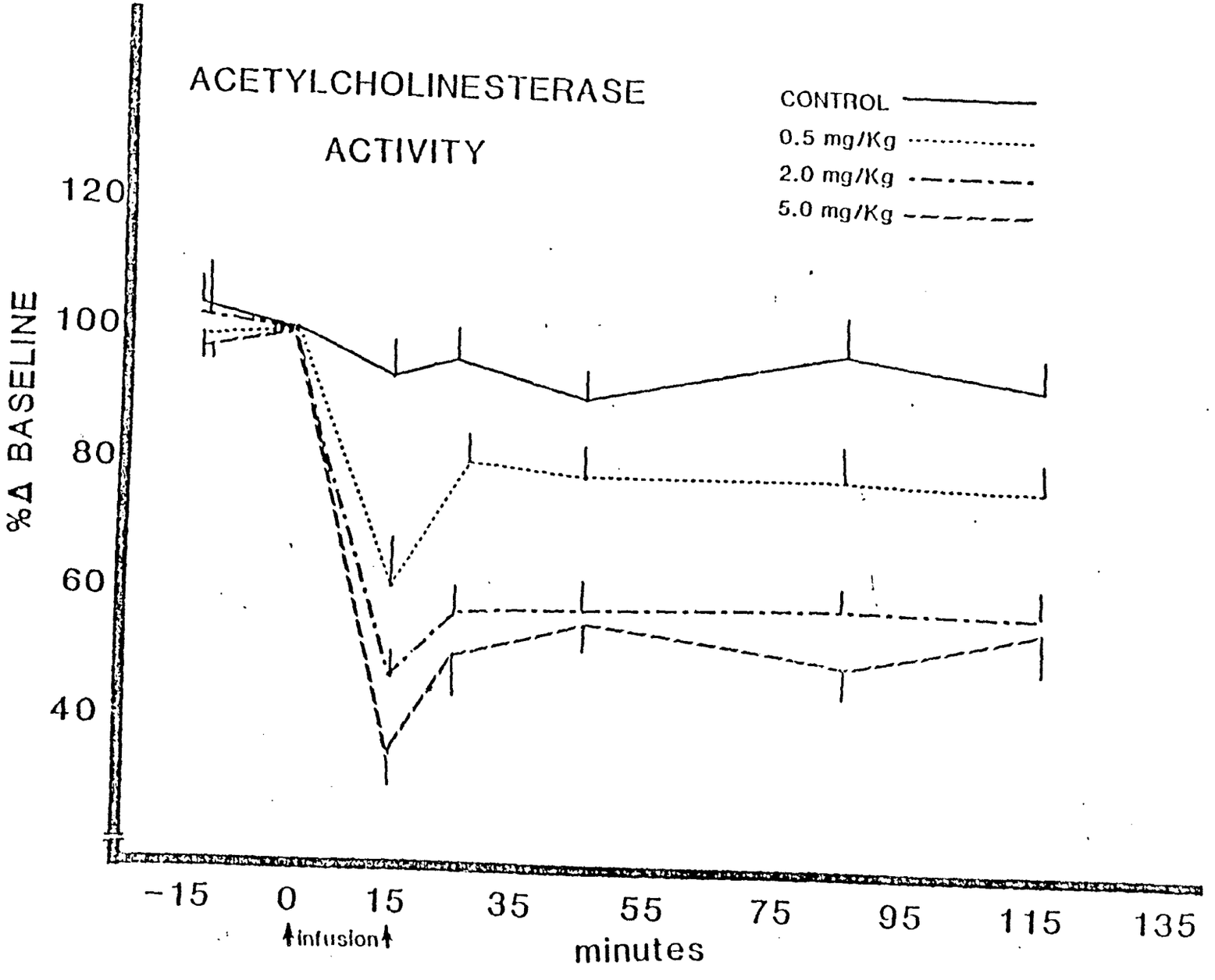

ACETYLCHOLINESTERASE
ACTIVITY
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
120
100
80
60
40
-15
0
15
35
55
75
95
115
135
Infusion
minutes

figure 15

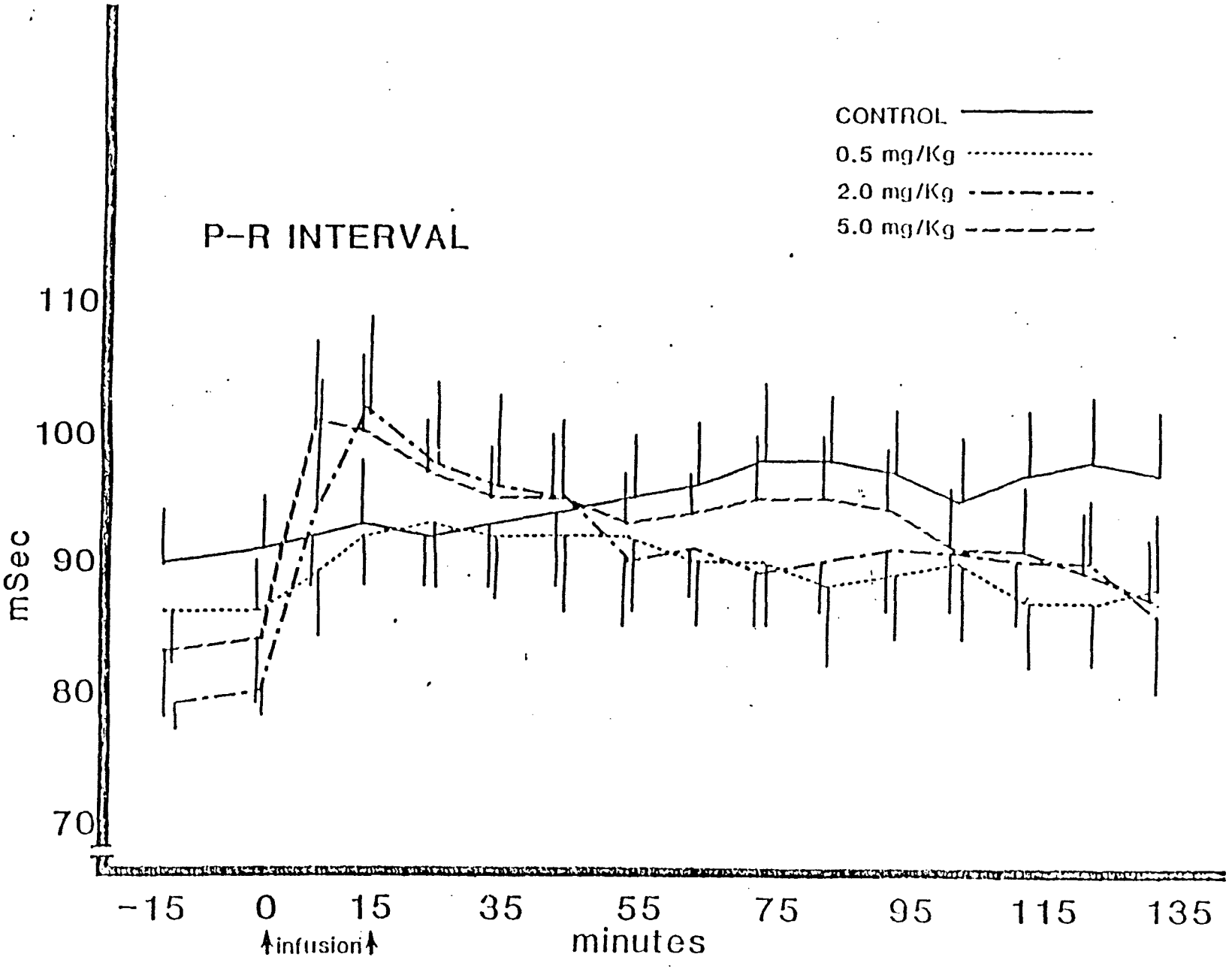
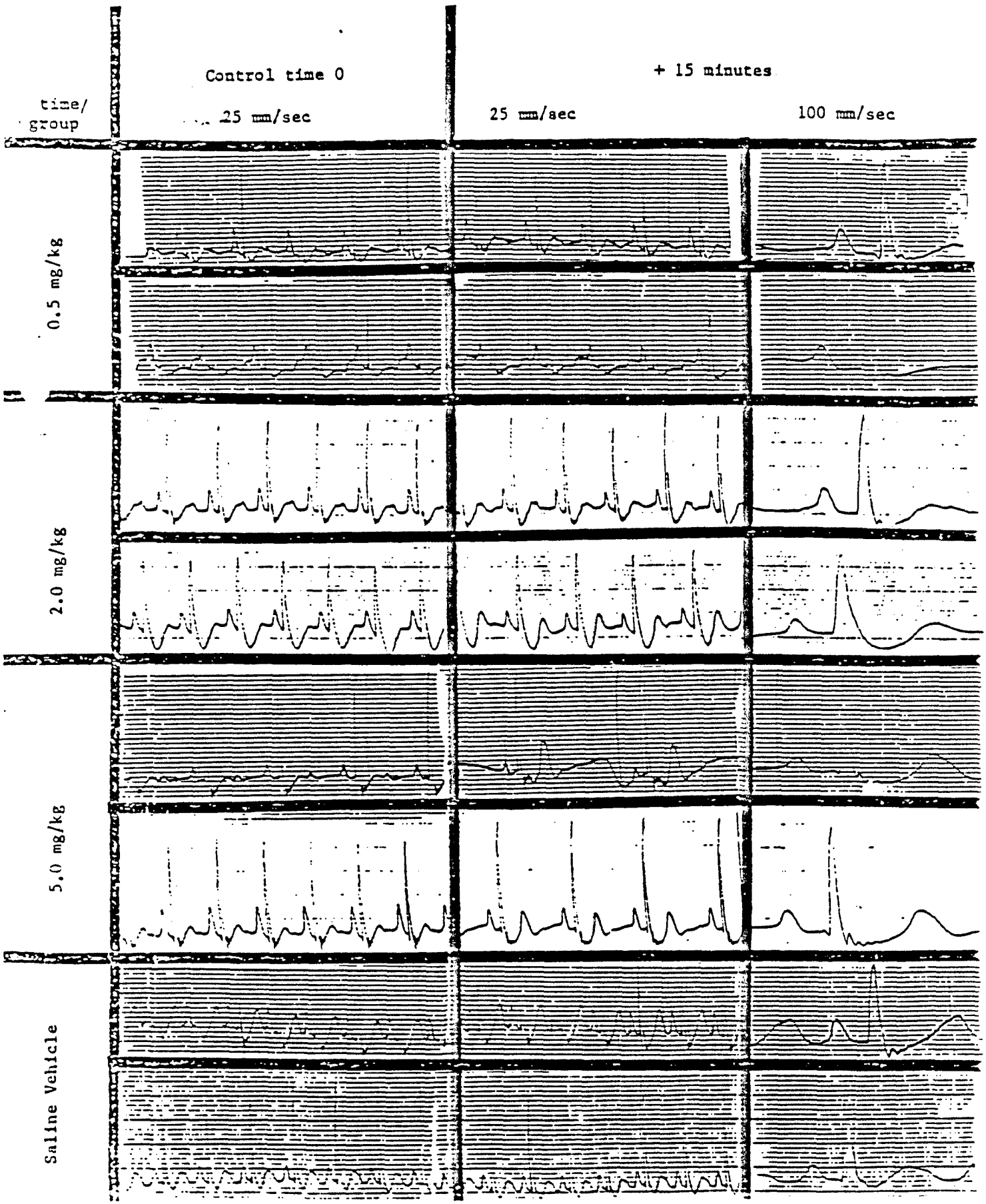

CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
P-R INTERVAL
110
100
90
80
70
mSec
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 16

Representative ECG tracings

Lead II



Appendix D

Composite Summaries - Beagles

Note: All variation bars represent S.E.M.

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChc	P-R
-15	410	9.2	3.77	66.8	2.2	124/85	129	20.7	2.67	2214	2.5	12.8	4.79	83.2	62.5	43.3	140.6	98
0	433	8.7	3.76	60.3	2.8	122/84	125	20.4	2.55	2235	2.5	11.9	4.67	88.4	61.8	41.0	**	98.
7.5	438	8.4	3.67	58.3	2.5	122/85	122	19.5	2.38	2133	1.5	12.0	5.04	96.2	61.7	42.5		98
15	470	8.4	3.94	58.5	2.7	123/86	123	19.9	2.45	2127	2.0	12.0	4.90	98.6	61.8	40.5	143.4	100
25	473	7.6	3.59	59.0	3.0	123/87	121	17.9	2.17	2136	5.5	11.9	5.48	95.4	61.0	*	128.1	99
35	525	6.4	3.36	60.8	3.1	127/89	120	19.2	2.30	2073	5.5	12.7	5.52					98
45	655	5.1	3.34	64.8	3.0	130/91	117	21.1	2.47	2064	6.0	13.3	5.38	97.5	58.9	40.0	141.6	100.
55	523	6.2	3.24	57.3	3.0	128/91	117	18.2	2.13	2106	5.5	12.3	5.77					99.
65	510	6.5	3.31	54.5	3.0	129/93	115	18.7	2.15	2265	5.0	12.2	5.67					100.
75	465	7.0	3.25	44.0	2.5	129/94	114	17.1	1.95	2343	5.0	12.0	6.15					101.
85	495	6.4	3.16	46.0	2.4	132/96	111	15.6	1.73	2367	4.5	12.7	7.34	97.4	61.4	39.5	133.2	103
95	493	7.1	3.50	43.3	2.3	131/95	111	17.2	1.91	2358	4.0	12.2	6.38					104
105	435	7.4	3.21	41.5	2.4	132/96	109	16.1	1.75	2308	4.0	11.3	6.46					104
115	418	7.3	3.05	41.0	2.0	132/97	107	16.4	1.75	2427	3.0	12.0	6.86	112.6	61.9	38.0	131.4	105
125	393	7.5	2.94	39.3	1.9	132/97	105	16.5	1.73	2418	2.5	12.1	7.00					106
135	410	7.6	3.11	38.9	1.6	130/96	104	15.9	1.65	2421	3.5	12.8	7.76					108

DRUG - Control

DOSE

WEIGHT - 13.2 kg

DATE - 9/10/85

*Hct tubes broken

** Blood sample lost

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo2	vPo2	Hct	AChe	P-R
-15	480	9.2	4.41	90.0	4.1	135/102	188	11.1	2.08	1437	2.5	11.9	5.72	74.4	50.3	*	310.9	78.5
0	488	9.1	4.44	85.6	4.5	134/107	180	9.6	1.72	1365	2.5	10.6	6.16	87.3	46.8	*	304.7	78.5
7.5	499	9.0	4.49	91.7	3.7	130/97	177	10.5	1.86	1368	3.5	8.8	4.73	72.5	45.0	*		78.5
15	521	8.6	4.48	109.9	3.3	126/99	170	10.6	1.81	1395	2.0	8.7	4.81	77.3	45.5	*	203.6	78.5
25	424	10.2	4.32	90.7	5.2	128/95	167	10.1	1.68	1479	2.0	9.1	5.42	72.4	45.2	*	240.2	79.0
35	464	9.0	4.17	88.4	5.3	131/96	171	11.0	1.88	1425	2.5	9.4	5.00					
45	503	8.5	4.27	90.0	3.7	129/96	174	10.7	1.86	1452	4.0	9.6	5.16	68.6	47.0	*	240.2	79.0
55	477	8.8	4.19	93.7	3.4	128/93	173	10.0	1.73	1503	3.5	9.8	5.66					
65	40	8.9	3.28	102.8	2.9	126/94	176	9.8	1.72	1509	4.0	9.9	5.76					
75	230	13.9	3.19	110.0	5.9	124/91	180	10.7	1.92	1542	3.0	11.7	6.09					
85	287	15.7	4.50	103.6	3.6	126/93	172	10.2	1.75	1482	3.5	10.6	6.06	89.5	54.9	*	246.7	79.5
95	327	14.1	4.61	93.8	3.8	127/94	167	10.0	1.67	1401	2.5	10.5	6.29					
105	383	12.7	4.86	94.1	4.2	126/93	164	9.5	1.56	1389	3.0	10.4	6.67					
115	358	14.6	5.22	97.3	5.4	125/94	162	10.3	1.67	1413	2.5	9.9	5.93	88.4	44.3	*	237.6	80.5
125	372	13.1	4.87	88.1	3.6	126/97	150	9.5	1.43	1363	2.5	9.8	6.85					
135	401	13.7	5.49	81.6	3.8	128/93	148	9.7	1.43	1329	2.5	10.4	7.27					

DRUG - Control DOSE *Hct tubes broken

WEIGHT - 9.7 kg DATE - 9/18/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh
-15	565	4.8	2.71	48	0.40	149/117	182	10.0	1.82	2037	1.5	13.7	7.53	77.7	56.0	41.5	180.
0	555	5.7	3.16	48	0.48	146/115	178	8.9	1.59	2034	2.0	11.5	7.23	86.1	57.8	40.8	181.
7.5	593	5.5	3.26	49	0.36	151/118	173	9.0	1.56	2037	1.5	11.7	7.50	91.4	52.1	40.0	
15	588	8.6	5.05	49	0.56	149/118	170	8.4	1.43	2085	7.5	10.4	7.27	69.2	33.7	38.8	168.
25	578	6.0	3.46	48	1.08	142/112	161	8.2	1.32	1932	5.5	9.4	7.12	86.4	48.7	38.0	180.
35	503	8.6	4.32	46	1.00	136/104	163	8.3	1.36	1875	3.5	8.9	6.54				
45	448	13.6	6.09	44	0.92	130/102	163	8.5	1.39	1887	4.5	6.4	4.60	89.0	38.2	39.5	158.
55	440	8.3	3.65	42	1.28	130/100	149	9.1	1.35	1761	4.0	5.7	4.22				
65	490	8.8	4.31	46	0.76	128/99	153	8.2	1.26	1740	1.5	5.7	4.52				
75	520	7.2	3.74	47	1.04	127/98	147	8.8	1.30	1794	2.5	4.5	3.46				
85	615	4.1	2.52	54	1.60	150/110	130	11.5	1.49	1938	3.5	9.0	6.04	48.6	34.7	39.0	172.
95	615	4.1	2.52	56	0.96	140/104	145	10.3	1.49	1824	1.5	9.2	6.17				
105	618	5.5	3.39	51	1.56	132/102	154	9.4	1.44	1779	2.0	8.0	5.56				
115	493	9.7	4.78	48	0.72	123/97	163	7.7	1.25	1740	2.0	6.2	4.96	93.1	43.7	39.5	168.
125	545	5.5	2.99	46	0.92	141/109	140	8.6	1.20	1761	3.0	7.0	5.83				
135	610	4.9	2.98	51	1.00	133/104	149	7.9	1.17	1611	1.0	6.3	5.38				

DRUG - Control DOSE

WEIGHT - 10.4 kg DATE - 9/25/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	dPo_2	vPo_2	Hct	ACh	
-15	651	3.1	2.01	67.5	1.6	129/92	113	19.8	2.24	1146	2.5	12.7	5.67	55.7	43.2	43.0	279.7	10
0	672	3.1	2.08	65.5	2.2	121/88	113	17.2	1.98	1119	3.5	8.7	4.39	64.7	41.2	41.5	225.8	10.
7.5	654	3.4	2.22	63.8	1.8	123/90	112	17.1	1.92	1047	6.0	8.2	4.27	65.1	30.0	42.0		10:
15	632	4.5	2.84	61.3	1.7	121/88	107	17.0	1.82	1089	3.5	7.8	4.29	86.1	47.8	40.5	225.6	10:
25	619	4.0	2.47	60.8	2.0	120/90	110	14.6	1.61	1089	6.5	7.4	4.60	98.5	46.9	40.0	223.5	104
35	570	3.6	2.05	59.4	2.0	117/88	120	14.4	1.73	1071	5.0	7.0	4.05					105
45	678	3.2	2.16	63.[illegible]	4.1	114/81	109	15.7	1.71	1011	5.0	8.0	4.68	85.9	48.0	39.5	221.0	107
55	626	4.0	2.50	61.0	3.2	104/75	109	14.1	1.54	942	3.5	6.7	4.35					106
65	631	4.2	2.65	60.7	2.5	100/71	108	12.2	1.32	855	5.5	6.3	4.77					108
75	691	1.7	1.17	81.9	2.5	108/76	99	18.5	1.83	864	5.5	7.8	4.26					113
85	703	1.6	1.12	82.5	2.0	157/98	92	28.6	2.63	1494	8.5	11.2	4.26	42.5	35.1	39.0	232.2	108.
95	701	1.9	1.33	83.1	2.7	154/97	96	26.5	2.54	1503	15.0	13.1	5.16					108.
105	701	1.5	1.05	84.5	1.6	146/92	95	24.8	2.36	1452	10.5	13.3	5.64					99.
115	704	1.6	1.12	85.1	1.5	156/91	89	28.9	2.57	1530	13.0	14.0	5.45	39.3	*	*	237.6	108.
125	707	2.0	1.41	89.6	1.0	142/85	85	30.0	2.55	1458	10.5	12.5	4.90					110.(
135	671	3.1	2.08	95.1	1.6	134/82	79	26.7	2.03	1344	13.0	9.9	4.88					109.(

DRUG - Control

DOSE

WEIGH - 10.0 kg

DATE - 10/1/85

* Venous blood sample broken

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACHe	P-R
-15	144	11.7	1.68	36.7	1.4	132/107	108	13.5	1.46	1638	5.0	10.4	7.12	68.9	43.8	32.0	299.1	92.5
0	95	24.0	2.28	40.6	0.9	123/100	108	13.3	1.44	1686	5.5	9.5	6.60	98.3	57.7	34.0	298.1	92.5
7.5	96	26.1	2.50	40.0	1.2	124/100	103	15.2	1.57	1686	5.0	10.2	6.50	70.7	44.7	20.0		92.5
15	93	26.3	2.44	40.1	1.2	126/100	103	15.0	1.54	1815	5.5	10.6	6.88	69.0	29.9	32.5	278.2	88.0
25	105	23.4	2.45	37.8	1.4	125/100	104	13.5	1.40	1920	4.0	10.0	7.14	64.4	29.8	45.9	290.8	88.0
35	107	18.2	1.94	31.9	1.5	124/97	102	13.9	1.42	1842	6.0	10.0	7.04					88.0
45	115	16.2	1.86	33.3	1.7	118/91	103	14.5	1.49	1872	5.0	9.8	6.58	93.7	47.7	41.0	289.0	88.0
55	96	21.3	2.04	32.6	1.5	*	97	15.4	1.49	1566	**	10.0	6.71					92.5
65	80	30.6	2.44	31.2	1.0	*	88	16.3	1.43	1383	**	11.6	8.11					93.0
75	88	27.3	2.40	31.3	1.7	*	88	15.1	1.33	1329	**	10.0	7.52					97.0
85	96	26.1	2.50	31.3	1.8	*	88	14.0	1.23	1374	**	8.9	7.24	87.7	38.0	35.8	282.0	97.5
95	107	21.6	2.31	35.3	1.8	*	87	11.7	1.02	1320	**	7.6	7.45					100.0
05	115	21.6	2.48	40.2	1.6	*	84	12.6	1.06	1281	**	7.2	6.79					101.5
15	116	27.2	3.15	37.0	1.3	125/100	93	11.3	1.05	1473	**	7.5	7.14	110.0	27.7	33.5	280.0	96.0
25	128	16.9	2.16	42.1	1.2	125/100	83	10.8	0.90	1197	**	6.6	7.33					97.0
35	151	13.3	2.00	43.0	†	130/105	82	11.3	0.93	1290	**	6.9	7.42					96.0

DRUG - Control

DOSE

WEIGHT - 10.1 kg

DATE - 10/15/85

* ABP transucer malfunctioning
**Swan-Ganz cathetes balloon broke
† Computer malfunction

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	P-
-15	132	12.4	1.63	18.2	6.4	190/105	143	14.2	2.03	3249	3.0	12.3	6.06	71.7	68.5	*	168.0	89.0
.0	124	13.6	1.68	17.7	5.4	160/90	127	11.3	1.44	2580	2.5	12.1	8.40	84.3	54.2	*	115.4	93.0
7.5	125	11.5	1.43	17.3	5.8	150/80	121	11.2	1.36	2577	2.5	11.8	8.68	82.2	49.1	*		96.0
15	130	11.9	1.54	17.5	5.1	150/80	120	10.8	1.30	2502	3.5	11.4	8.77	84.9	48.8	*	176.9	100.0
25	127	11.6	1.47	16.8	5.3	155/85	119	10.7	1.27	2559	3.5	10.5	8.27	87.1	45.1	*	185.9	99.0
35	118	17.9	2.11	17.6	5.8	140/75	118	10.5	1.24	2193	3.5	8.5	6.85					102.5
45	117	17.5	2.04	17.2	6.4	130/70	114	10.4	1.18	2103	3.5	7.5	6.36	90.1	41.4	*	126.5	107.0
55	116	19.8	2.29	17.4	6.3	140/75	113	10.2	1.15	1968	3.5	6.9	6.00					108.0
65	110	25.3	2.78	16.9	6.1	130/70	113	9.4	1.06	2007	1.5	6.7	6.32					108.0
75	109	16.5	1.79	17.8	5.8	135/70	109	10.5	1.14	1941	3.0	7.6	6.67					109.0
85	110	11.5	1.26	17.5	4.9	145/75	104	12.8	1.33	2049	2.5	9.3	6.99	79.7	46.6	*	202.3	106.0
95	128	7.5	0.96	17.3	5.3	145/75	107	13.8	1.48	2079	5.0	12.4	8.37					103.0
105	134	8.7	1.16	16.8	5.3	**	108	12.0	1.30	2142	5.0	11.8	9.08					103.0
115	134	9.2	1.23	17.3	4.8	**	107	12.2	1.31	2196	5.5	11.1	8.47	89.0	58.8	*		104.0
125	134	9.5	1.27	17.7	4.8	160/85	105	11.8	1.24	2016	5.5	10.4	8.39					104.0
135	130	10.0	1.30	17.3	4.5	155/85	106	10.9	1.16	2127	4.5	10.4	8.97					104.0

DRUG - Control

DOSE

WEIGHT - 9.8 kg

DATE - 11/20/85

* Hct tubes broken
**ADR transducer malfunctioning
† Sample tube broken

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	P-
-15	144	9.8	1.41	26.1	6.3	187/146	172	17.6	3.02	1755	6.0	21.3	7.05	60.2	50.5	*	339.1	71
0	143	9.6	1.37	26.3	6.4	190/149	168	17.5	2.94	1746	4.0	17.2	5.85	50.1	48.3	*	374.5	71
7.5	141	9.3	1.31	24.9	6.9	195/149	172	17.6	3.03	1971	4.0	16.1	5.31	51.7	55.1	*		71
15	136	8.6	1.16	23.9	7.4	199/146	153	22.0	3.37	2073	4.5	16.3	4.84	67.8	51.1	*	242.8	76
25	140	8.3	1.16	23.5	7.6	199/147	153	20.1	3.08	2091	6.5	15.3	4.97	69.5	56.4	*	298.0	76
35	143	7.2	1.02	24.3	8.1	195/151	155	19.0	2.95	2019	3.0	14.8	5.02					76
45	149	7.1	1.05	24.8	7.6	196/154	154	17.2	2.65	1932	2.0	13.7	5.17	60.2	54.8	*	308.9	76
55	148	6.6	0.97	24.5	8.2	185/148	152	15.5	2.36	1695	2.0	12.4	5.25					76
65	149	7.1	1.05	25.3	8.3	178/143	151	14.1	2.13	1608	2.0	11.7	5.49					76
75	150	7.3	1.09	25.0	9.0	175/140	149	14.2	2.12	1602	1.5	11.5	5.42					76
85	140	6.6	0.92	24.3	8.5	176/140	145	14.2	2.06	1575	1.5	11.3	5.46	62.9	59.0	*	329.2	72
95	124	7.0	0.86	24.0	6.7	184/145	143	16.5	2.36	1722	1.5	12.0	5.08					72
105	125	7.6	0.95	25.1	6.0	188/148	146	15.5	2.26	1833	1.5	12.0	5.31					72
115	121	8.3	1.00	25.5	6.3	185/145	146	15.2	2.22	1821	1.5	11.4	5.14	58.0	33.3	*	300.7	72
125	117	8.6	1.00	25.6	6.2	174/136	142	14.6	2.07	1665	1.0	10.6	5.12					72
135	115	8.7	1.00	25.3	6.1	170/132	140	14.0	1.96	1661	1.0	10.3	5.26					72

DRUG - Pyridostigmine DOSE - 0.5 mg/kg *Hct tubes broken

WEIGHT - 12.3 kg DATE - 8/14/85 ** Blood gas machine malfunctioning

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACho	P-R.
-15	540	5.3	2.86	83.8	2.8	130/104	135	15.5	2.09	1287	5.0	8.7	4.16	64.2	47.1	45.0	282.9	87
0	480	6.3	3.02	87.8	2.7	131/95	129	14.7	1.90	1356	3.0	8.9	4.68	70.4	47.1	44.0	285.6	88
7.5	385	6.0	2.31	82.8	3.5	135/93	134	13.7	1.84	1368	7.0	12.2	6.63	73.3	45.6	43.5		88
15	435	8.1	3.52	83.8	4.0	136/88	115	17.7	2.04	1395	12.0	13.4	6.57	77.6	54.7	44.5	*	96
25	458	7.0	3.20	80.0	4.2	133/85	111	16.8	1.86	1410	6.0	12.9	6.93	84.8	56.3	45.0	276.0	94
35	473	6.7	3.16	76.5	4.4	128/89	127	14.3	1.81	1446	5.0	11.8	6.52					93.5
45	520	6.3	3.27	77.0	4.8	127/90	128	14.6	1.87	1356	6.0	11.5	6.15	85.8	49.1	44.0	272.8	88
55	488	6.8	3.31	79.0	4.8	125/87	125	14.1	1.76	1344	4.0	10.9	6.19					89
65	513	7.3	3.74	82.8	4.9	127/90	128	12.0	1.54	1359	3.0	10.1	6.56					87.5
75	565	6.7	3.78	79.5	5.9	125/94	126	13.2	1.66	1287	5.0	9.9	5.96					88
85	603	5.9	3.55	75.0	4.4	127/96	129	11.9	1.53	1245	4.0	10.2	6.67	73.0	40.7	44.5	262.5	87.5
95	630	4.0	2.52	74.3	4.6	126/87	92	15.2	1.40	1077	4.0	12.6	9.08					91
105	658	4.1	2.69	70.5	4.7	130/89	93	14.2	1.32	1017	5.0	12.3	9.32					93.5
115	680	4.5	3.06	73.3	4.2	130/94	105	12.3	1.29	1032	6.5	12.0	9.30	87.8	47.3	44.5	269.3	87.5
125	730	4.6	3.35	74.3	4.0	132/99	126	10.2	1.29	1239	4.0	12.6	9.77					86
135	660	5.8	3.82	85.3	2.8	135/102	122	10.9	1.33	1209	3.0	11.5	8.65					85

DRUG - Pyridostigmine DOSE - 0.5 mg/kg *Sample tube broken

WEIGHT - 12.3 kg DATE - 9/17/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	P-R
-15	166	8.0	1.32	55.5	1.2	129/108	140	8.1	1.14	2508	1.5	6.5	5.70	91.2	51.5	32.0	210.7	95
0	153	10.1	1.53	57.3	1.4	115/92	142	8.9	1.27	2328	1.5	4.9	3.86	100.0	49.1	33.0	247.8	95
7.5	157	8.8	1.38	55.5	1.3	126/100	138	9.1	1.26	2472	2.5	5.3	4.21	99.4	48.4	34.0		98
15	149	8.8	1.31	55.8	2.1	128/93	126	11.8	1.49	2631	3.0	5.8	3.89	105.8	*	*	130.9	101
25	118	9.5	1.12	53.5	1.9	121/86	122	11.6	1.42	2472	1.5	6.1	4.30	92.9	58.8	37.5	165.5	101
35	134	8.6	1.15	55.7	1.9	123/90	119	11.5	1.37	2427	2.0	6.5	4.74					102
45	144	8.8	1.26	53.5	1.8	119/83	122	11.6	1.42	2529	3.5	6.4	4.51	98.7	57.2	36.5	169.0	104
55	145	9.0	1.30	58.7	2.1	121/87	125	11.1	1.39	2541	1.5	5.8	4.17					103
65	142	10.2	1.44	58.9	2.4	121/88	125	11.7	1.46	2592	4.0	5.5	3.77					101
75	145	9.8	1.42	58.4	4.3	119/87	128	10.6	1.36	2604	4.5	6.3	4.63					101
85	151	9.2	1.38	57.3	3.3	119/87	129	9.5	1.23	2457	3.5	5.8	4.72	101.5	57.3	35.5	181.7	102
95	158	7.1	1.12	63.7	4.7	115/85	122	10.7	1.31	2499	1.5	6.1	4.66					98
105	162	7.7	1.24	65.0	4.4	115/82	124	11.0	1.37	2565	3.5	6.2	4.53					99
115	161	8.2	1.32	62.6	6.1	106/74	125	11.4	1.43	2478	2.5	5.6	3.92	108.2	57.9	34.0	172.4	99
125	158	7.1	1.12	65.2	4.9	113/79	119	11.3	1.35	2550	1.5	5.9	4.37					99
135	163	6.6	1.07	61.9	3.4	114/79	119	12.0	1.43	2556	3.0	5.9	4.13					100

DRUG - Pyridostigmine DOSE - 0.5 mg/kg *Venous blood sample tube broken

WEIGHT - 12.5 kg DATE - 10/22/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	P-R
-15	74	48.4	3.58	20.5	2.3	184/123	178	8.7	1.54	3444	3.0	11.5	7.47	82.7	23.6	*	233.7	82.3
.0	68	43.9	2.98	18.4	2.8	189/128	164	7.9	1.30	3363	2.0	10.1	7.77	85.8	20.6	*	227.2	81.0
7.5	66	46.5	3.06	17.8	2.8	178/129	171	7.7	1.32	3348	1.5	10.7	8.10	90.1	33.3	*		81.0
15	78	47.7	3.72	19.1	4.7	167/103	169	8.4	1.42	3306	3.5	11.6	8.17	98.0	27.5	*	97.5	83.5
25	60	41.6	2.49	15.8	11.5	186/120	155	7.3	1.13	3213	4.5	10.6	9.38	92.2	32.3	*	150.5	86.0
35	68	43.8	2.97	16.2	8.1	181/116	157	7.8	1.22	3021	1.0	8.0	6.55					80.0
45	77	45.8	3.52	16.6	3.5	177/114	167	7.3	1.22	3066	1.0	7.9	6.47	91.7	23.1	*	150.0	79.0
55	73	45.4	3.31	16.7	3.2	185/119	167	6.4	1.07	3072	1.0	7.2	6.72					79.0
65	84	45.4	3.81	17.2	2.6	190/128	173	6.7	1.16	2979	3.0	12.4	10.69					76.0
75	78	41.7	3.25	17.1	1.9	199/138	172	6.1	1.05	3012	2.5	11.1	10.57					76.0
85	84	42.1	3.53	16.6	2.1	206/138	177	6.8	1.21	3183	2.5	11.8	9.75	95.1	25.6	*	124.6	76.0
95	84	44.5	3.73	16.4	3.0	201/134	182	6.2	1.12	3231	2.5	12.0	10.71					76.0
105	74	57.2	4.23	17.1	3.6	192/129	173	6.4	1.11	3042	5.5	12.7	11.44					76.0
115	72	51.4	3.70	16.9	3.4	194/130	177	5.8	1.03	3087	2.5	12.1	11.74	82.2	31.0	*	150.7	77.5
125	77	52.8	4.06	17.2	2.9	187/128	189	5.6	1.06	2907	1.5	13.7	12.92					77.0
135	83	53.2	4.41	17.0	3.0	187/128	192	5.7	1.10	3078	2.0	14.9	13.54					77.5

DRUG - Pyridostigmine DOSE - 0.5 mg/kg *Hct tubes broken

WEIGHT - 8.6 kg DATE - 10/29/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh$_c$	P-R
-15	260	11.3	2.93	51.8	2.7	190/145	183	11.4	2.09	3150	4.0	14.8	7.08	82.5	42.5	42.5	157.0	94.0
0	261	11.8	3.07	54.9	3.0	185/145	194	10.7	2.08	3225	4.0	14.6	7.02	77.4	46.1	46.0	152.1	93.5
7.5	289	10.6	3.06	48.1	3.7	185/140	183	11.5	2.11	3165	7.5	15.8	7.49	77.9	46.1	46.5		94.0
15	243	19.1	4.64	51.3	5.3	180/130	174	14.9	2.59	3294	7.5	16.1	6.22	79.2	46.6	48.0	111.6	95.0
25	231	12.5	2.88	47.2	13.8	170/125	196	11.7	2.30	3054	7.0	14.7	6.39	64.0	34.6	46.5	136.0	95.0
35	371	9.9	3.67	46.2	13.6	155/115	169	11.6	1.96	2775	8.5	13.4	6.84					95.0
45	470	7.2	3.38	40.2	32.0	160/115	147	12.4	1.83	2463	10.0	13.7	7.49	66.9	35.2	44.0	147.2	102.0
55	474	7.4	3.50	38.1	20.2	155/115	150	13.1	1.97	2424	4.5	14.1	7.16					102.0
65	440	8.3	3.65	41.5	16.6	155/120	168	11.0	1.84	2436	6.5	14.9	8.10					100.0
75	486	7.4	3.59	38.9	20.8	150/120	163	11.4	1.86	2361	5.0	15.1	8.12					96.0
85	448	9.4	4.21	42.1	24.8	150/120	184	8.8	1.61	2526	2.0	15.0	9.32	7.11	36.4	*	146.7	92.0
95	490	6.5	3.18	42.6	20.8	155/125	176	8.2	1.44	2259	7.0	14.9	10.35					92.0
105	463	8.3	3.84	40.6	16.1	150/120	180	8.6	1.55	2328	7.0	14.7	9.48					91.5
115	387	12.6	4.87	42.3	8.3	165/130	195	8.4	1.64	2535	4.5	14.7	8.96	79.4	31.5	44.0	146.5	81.0
125	366	13.0	4.75	42.8	7.0	145/115	200	7.6	1.51	2472	8.0	14.3	9.47					88.0
135	322	14.4	4.63	41.5	5.2	150/115	203	7.6	1.54	2463	12.0	17.7	11.49					84.5

DRUG - Pyridostigmine DOSE - 0.5 mg/kg *Hct tube broken

WEIGHT - 13.4 kg DATE - 11/13/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PMP	PAP	PVR	aPo_2	vPo_2	Hct	ACho	P-R
-15	203	5.3	1.07	19.2	4.3	160/110	151	11.3	1.70	2523	2.5	12.4	7.29	70.9	47.8	42.0	194.3	89.
.0	239	4.6	1.09	19.2	3.8	155/100	145	11.4	1.66	2349	3.0	11.5	6.93	68.2	56.0	39.5	173.5	89.
7.5	262	3.8	0.99	17.3	6.8	150/95	125	15.9	1.99	2073	3.5	11.3	5.68	56.5	51.9	41.0		100.
15	218	4.8	1.04	17.2	6.9	145/80	112	11.6	1.30	1878	3.5	11.4	8.77	74.4	53.5	44.0	112.0	100.
25	184	6.0	1.10	17.4	6.3	125/75	97	13.3	1.29	1818	1.5	7.6	5.89	78.8	49.3	42.0	111.0	104.
35	182	7.3	1.32	17.5	6.4	125/75	95	13.6	1.29	1524	2.5	7.5	5.81					104.
45	165	9.5	1.56	17.4	5.9	125/75	101	14.9	1.50	1587	1.5	7.2	4.80	80.5	38.2	39.0	102.1	104.
55	143	12.2	1.74	17.3	5.5	120/80	103	11.4	1.17	1593	1.5	7.0	5.98					102.
65	141	11.9	1.67	17.0	5.1	115/75	108	11.2	1.21	1515	2.5	6.6	5.45					101.
75	134	13.2	1.76	16.8	5.0	120/80	105	13.3	1.40	1470	2.0	6.4	4.57					101.
85	129	14.7	1.89	16.6	4.8	125/85	112	11.5	1.29	1491	2.5	6.3	4.88	107.7	50.4	39.0	111.4	100.
95	117	13.2	1.54	16.9	3.8	110/70	97	11.6	1.13	1221	2.0	6.0	5.31					103.
105	155	8.0	1.24	15.9	5.3	125/80	93	11.1	1.03	1383	2.5	6.9	6.69					105.
115	161	7.3	1.17	15.7	5.2	130/80	97	11.3	1.10	1434	1.5	7.0	6.36	88.1	48.4	39.0	96.9	103.
125	160	7.2	1.15	15.6	5.2	130/80	94	8.2	0.77	1365	3.0	6.4	8.31					102.
135	164	6.7	1.09	15.2	5.3	130/85	96	8.8	0.85	1365	3.0	6.5	7.65					106.

DRUG - Pyridostigmine DOSE - 0.5 mg/kg

WEIGHT - 8.2 kg DATE - 11/27/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	
-15	143	11.7	1.67	21.2	2.1	123/93	156	10.0	1.56	2250	3.0	11.0	7.05	78.0	47.2	41.5	181.6	7
0	133	10.8	1.43	20.1	2.0	120/91	148	9.1	1.34	2037	3.0	10.0	7.46	93.9	40.6	40.5	199.8	7
7.5	143	15.6	2.23	20.2	4.5	132/95	145	11.4	1.65	2478	2.5	11.3	6.85	86.0	39.5	37.0		8
15	154	15.8	2.43	26.8	5.2	132/91	127	11.3	1.44	2805	3.0	11.0	7.64	84.0	43.0	44.5	74.6	9
25	119	11.6	1.38	16.5	4.9	122/87	115	11.0	1.27	2031	2.5	9.8	7.72	77.9	38.2	44.5	93.8	9
35	118	12.4	1.46	18.9	4.9	129/94	119	10.6	1.26	2118	2.0	9.4	7.46					9
45	134	13.4	1.79	19.1	8.1	132/97	121	11.2	1.36	2163	3.0	9.3	6.84	85.5	37.4	29.0	100.3	9
55	125	15.8	1.97	18.6	8.4	131/98	121	11.8	1.43	2106	3.0	9.6	6.71					8
65	111	8.7	0.96	16.4	7.0	154/109	111	13.8	1.53	2454	3.0	13.6	8.89					8
75	104	12.7	1.32	16.4	7.7	154/111	118	13.5	1.59	2649	3.0	13.2	8.30					8
85	101	12.8	1.29	15.9	9.3	154/114	118	11.8	1.39	2631	2.0	14.0	10.07	84.4	47.1	40.0	94.5	8
95	104	13.7	1.42	16.3	10.9	150/114	122	10.3	1.26	2505	2.0	11.7	8.67					8
105	118	14.8	1.74	20.5	10.8	150/115	123	11.0	1.35	2604	2.0	11.6	8.59					8
115	106	9.0	0.95	15.3	9.4	169/121	115	13.1	1.51	2997	4.0	17.8	11.79	70.9	52.3	41.0	99.6	8
125	109	10.3	1.12	15.5	10.0	165/123	118	10.7	1.26	2973	2.0	12.6	10.00					8
135	112	10.0	1.12	15.0	12.3	163/124	117	10.9	1.28	2754	2.5	12.9	10.01					8

DRUG - Pyridostigmine DOSE - 2.0 mg/kg *pH electrode malfunctioning

WEIGHT - 12.3 kg DATE - 8/7/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	P-
-15	123	15.1	1.85	27.0	4.5	146/112	172	14.4	2.48	1710	6.0	15.8	6.37	69.9	60.0	42.0	142.8	73
,0	120	15.1	1.81	26.3	4.5	152/116	166	13.9	2.30	1779	5.0	15.9	6.91	83.0	66.1	43.0	160.8	73
7.5	116	13.6	1.57	24.0	6.6	175/124	172	15.1	2.60	2205	6.0	19.8	7.62	81.2	66.3	49.5		73
15	96	18.1	1.73	22.8	6.8	182/130	146	20.3	2.96	2130	11.0	23.4	7.90	77.1	70.2	51.5	60.4	90
25	88	19.2	1.68	22.4	7.3	172/125	148	18.0	2.66	2052	5.0	15.1	5.68	86.4	61.7	53.0	82.8	87
35	77	19.5	1.50	23.9	10.6	150/117	130	13.4	1.74	1449	3.5	10.0	5.75					87
45	84	19.1	1.60	24.7	8.7	145/115	129	14.7	1.89	1416	5.0	11.2	5.92	88.2	60.4	49.5	85.4	83
55	74	19.7	1.45	18.8	16.7	146/107	129	15.3	1.97	1431	8.0	10.5	5.33					82
65	85	17.4	1.47	19.7	11.1	156/112	134	17.8	2.39	1635	5.0	12.2	5.10					82
75	90	15.7	1.41	18.6	11.3	157/115	137	16.4	2.25	1638	3.5	11.4	5.07					79
85	94	15.0	1.41	18.3	10.8	151/113	130	14.9	1.94	1446	4.0	9.8	5.05	91.8	*	48.0	91.7	79
95	90	15.5	1.39	18.3	9.4	140/110	123	12.9	1.59	1245	1.0	7.9	4.97					79
105	98	15.2	1.48	18.1	9.3	133/106	117	11.9	1.39	1083	3.0	6.6	4.75					80
115	105	14.4	1.51	17.9	9.2	132/108	115	11.6	1.33	1059	6.0	7.1	5.34	110.4	54.1	48.0	89.3	81
125	111	13.1	1.45	18.0	10.2	134/109	116	10.5	1.22	951	3.0	6.8	5.57					8.
135	116	12.7	1.47	20.8	9.6	137/110	119	11.0	1.31	1176	4.0	6.5	4.96					7.

DRUG - Pyridostigmine DOSE - 2.0 mg/kg *equipment malfunction

WEIGHT - 10.3 kg DATE - 8/19/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	
-15	154	11.0	1.69	36.6	1.6	155/115	135	15.3	2.07	2181	2.0	7.6	3.67	91.7	55.5	42.0	241.1	
0	157	10.9	1.71	36.4	1.4	130/100	135	16.1	2.17	2157	1.0	7.2	3.32	99.8	55.7	40.5	23.50	
7.5	149	9.6	1.43	34.4	5.4	125/95	113	19.9	2.25	2214	**	8.7	3.87	62.2	50.4	37.5		1
15	48	59.8	2.87	25.1	6.5	115/80	99	16.4	1.62	1872	**	12.0	7.41	41.3	35.3	38.0	148.0	1
25	77	38.7	2.97	37.2	6.8	105/75	80	16.9	1.35	1203	**	10.5	7.78	61.1	43.9	39.5	176.0	1
35	76	39.4	2.99	52.0	6.4	100/70	81	15.9	1.29	1257	**	9.8	7.60					1
45	78	40.1	3.12	42.1	8.0	105/80	89	15.6	1.39	1974	6.0	9.8	7.05	107.0	46.0	39.5	163.4	1
55	92	39.4	3.62	36.5	9.2	90/65	96	14.6	1.40	1422	3.5	9.3	6.64					1
65	95	20.4	1.93	45.3	7.3	105/80	91	16.8	1.53	1077	3.0	7.0	4.58					1
75	117	18.5	2.16	28.0	7.8	100/75	109	13.4	1.46	*	1.0	5.2	3.56					1
85	120	20.2	2.42	27.0	7.2	120/80	104	12.9	1.34	*	**	3.7	2.76	120.0	41.0	40.0	149.4	1
95	116	9.9	1.14	28.9	8.1	130/80	95	15.0	1.42	*	1.0	4.6	3.24					1
105	122	10.5	1.28	33.1	7.8	135/90	91	14.8	1.35	2058	3.0	7.2	5.33					10
115	121	13.9	1.68	30.2	7.8	130/85	95	12.4	1.18	1938	2.0	6.3	5.34	112.2	50.0	41.5	138.0	10
125	117	18.0	2.10	30.5	5.5	125/85	100	11.3	1.13	1293	2.5	5.6	4.96					10
135	120	19.8	2.37	30.3	5.5	125/85	101	12.6	1.27	1275	1.5	5.4	4.25					10

DRUG - Pyridostigmine DOSE - 2.0 mg/kg

WEIGHT - 12.2 kg DATE - 10/9/85

*Millar® catheter position altered

**Swan Ganz catheter balloon broke

† P-R Interval not recor

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	
-15	162	4.5	0.72	12.9	2.6	177/146	136	9.7	1.32	2103	1.5	12.8	9.70	75.7	48.7	*	202.2	
0	177	5.2	0.92	13.0	2.3	177/147	154	8.4	1.29	2235	3.0	12.1	9.38	69.5	54.5	*	212.6	
7.5	55	25.3	1.39	12.2	8.8	185/146	167	18.8	3.14	2583	4.0	13.4	4.27	87.8	41.9	*		
15	104	20.4	2.12	9.9	10.5	216/160	139	13.3	1.85	3183	4.0	15.7	8.49	78.6	46.3	*	85.0	
25	154	10.8	1.66	12.0	10.1	185/139	132	11.5	1.52	2898	5.0	12.3	8.09	81.0	45.3	*	96.5	
35	124	11.8	1.46	16.2	8.4	154/111	139	12.9	1.79	2403	3.0	10.1	5.64					
45	136	12.3	1.67	15.0	8.4	143/109	130	10.7	1.39	1773	4.0	8.2	5.90	85.1	47.9	*	89.1	
55	141	21.1	2.97	16.6	8.2	150/111	146	14.3	2.08	2115	3.0	8.8	4.23					
65	138	12.7	1.75	14.2	7.8	159/114	133	13.7	1.82	2145	2.5	9.0	4.94					
75	164	12.5	2.05	15.2	8.8	154/110	140	9.3	1.30	2046	2.5	8.8	6.77					
85	165	9.6	1.58	13.8	9.1	152/112	144	11.6	1.67	1977	2.5	8.0	4.79	68.2	41.0	*	100.2	
95	174	10.0	1.74	14.9	8.1	141/105	143	10.2	1.46	1869	1.5	7.5	5.13					
105	157	18.8	2.95	15.1	7.5	135/99	141	5.3	0.75	1785	5.0	7.5	10.0					
115	153	12.2	1.86	14.6	7.6	121/89	136	7.1	0.96	1698	3.0	8.4	8.75	83.3	49.2	*	119.5	
125	151	15.9	2.40	15.0	8.1	131/98	143	6.5	0.93	1830	1.5	9.0	9.68					
135	159	19.2	3.05	16.1	6.5	138/104	154	8.1	1.25	2061	1.0	9.4	7.52					

DRUG - Pyridostigmine DOSE - 2.0 mg/kg *Hct tubes broken

WEIGHT - 8.6 kg DATE - 10/30/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	I
-15	102	7.1	0.72	18.2	2.8	145/105	155	5.6	0.86	2619	3.0	14.7	17.1	62.2	39.9	**	136.8	8
0	101	7.3	0.73	18.0	2.7	135/95	156	5.3	0.82	2727	3.5	14.9	18.2	80.6	48.8	**	100.3	8
7.5	107	6.5	0.69	16.6	4.6	140/90	135	6.5	0.88	2982	†	17.6	20.0	86.8	59.5	**		9
15	119	7.3	0.86	17.0	5.6	155/90	118	6.4	0.75	3591	4.0	17.3	23.0	93.2	58.6	**	54.4	10
25	100	9.1	0.91	18.3	4.6	140/100	137	6.0	0.82	3459	2.0	12.9	15.7	95.9	63.9	**	61.2	9
35	95	10.1	0.95	18.2	5.5	*	141	4.4	0.62	2640	5.0	9.7	15.6					9
45	100	11.7	1.17	18.9	5.2	*	144	3.9	0.56	2277	4.0	9.5	17.0	111.7	35.3	**	68.2	9
55	100	13.3	1.33	18.1	4.9	*	145	3.8	0.55	2067	4.5	9.2	16.7					8
65	97	14.7	1.42	18.5	5.8	*	136	4.8	0.65	1878	4.5	8.8	13.5					9
75	94	15.0	1.41	18.1	7.1	*	125	4.3	0.54	1605	5.0	8.5	15.7					9
85	87	17.3	1.50	17.5	7.2	105/70	120	4.5	0.54	1578	3.5	8.1	15.0	108.0	27.2	**	68.3	9
95	83	15.7	1.30	16.3	7.1	105/75	109	4.4	0.48	1470	3.0	7.6	15.8					9
105	81	17.2	1.39	16.8	6.9	105/75	104	4.3	0.45	1410	4.0	7.5	16.7					9
115	82	16.5	1.35	16.5	7.1	110/75	97	4.1	0.40	1398	2.5	7.2	18.0	109.1	27.3	**	70.0	9
125	84	15.4	1.29	16.6	6.6	110/70	93	4.6	0.43	1380	3.5	7.2	16.7					9
135	82	17.7	1.45	16.6	7.0	110/75	107	4.1	0.44	1497	3.5	6.9	15.7					9

DRUG - Pyridostigmine

DOSE - 2.0 mg/kg

WEIGHT - 6.7 kg

DATE - 11/5/85

*Instrument malfunction

**Hct tubes broken

†Swan Ganz catheter position alter

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	
-15	161	6.7	1.07	25.3	3.9	170/125	137	11.8	1.61	3033	3.5	14.3	8.88	45.4	29.7	39.0	154.9	
0	161	6.6	1.06	27.0	3.8	165/120	137	10.7	1.46	3078	3.5	9.5	6.51	82.7	40.3	38.0	162.2	
7.5	123	9.9	1.21	25.0	5.9	150/100	123	12.9	1.59	3126	3.0	10.9	6.86	92.8	60.5	40.0		
15	92	10.6	0.97	18.0	7.0	175/105	105	16.4	1.72	3135	7.0	15.1	8.78	75.2	58.8	48.5	72.9	
25	95	11.2	1.06	18.2	11.2	165/105	112	14.2	1.59	3402	7.5	13.5	8.49	109.4	61.9	44.5	90.0	
35	94	13.3	1.25	20.2	5.9	155/95	124	12.9	1.60	3357	1.5	9.6	6.00					
45	103	13.4	1.38	20.3	4.9	155/100	132	11.9	1.57	3483	2.5	9.8	6.24	88.4	59.2	53.4	91.9	
55	98	13.8	1.35	21.3	5.1	150/90	130	11.5	1.50	3258	3.5	7.3	4.87					
65	93	10.0	0.93	20.4	5.4	145/95	126	12.5	1.58	3129	3.5	7.9	5.00					
75	119	5.0	0.59	19.6	6.0	150/90	115	13.2	1.52	2898	3.5	10.0	6.58					
85	137	4.2	0.57	19.7	6.9	145/90	101	15.9	1.61	2634	2.5	10.5	6.52	66.6	36.4	40.5	101.0	
95	147	4.5	0.66	19.1	7.6	140/90	97	14.6	1.42	2310	1.5	10.0	7.04					1
105	155	3.9	0.60	18.6	7.6	135/80	89	15.2	1.35	2124	3.0	10.5	7.78					1
115	168	3.5	0.58	18.8	9.0	125/75	88	14.4	1.27	1995	4.0	9.7	7.64	60.6	36.8	39.0	**	1
125	166	3.7	0.61	18.4	9.0	*	94	12.6	1.18	1992	3.0	8.7	7.37					1
135	174	3.1	0.53	19.1	10.0	130/75	88	13.4	1.18	2016	3.5	14.0	11.86					

DRUG - Pyridostigmine

DOSE - 2.0 mg/kg

WEIGHT - 8.5 kg

DATE - 11/12/85

*equipment malfunctioning
**assay sample tube broken

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PMP	PAP	PVR	aPo_2	vPo_2	Hct	ACh
-15	185	6.0	1.11	30.0	8.2	147/112	154	15.6	2.41	2907	4.5	16.5	6.85	66.3	59.4	41.0	88.9
0	195	6.3	1.22	30.1	8.3	147/115	154	17.5	2.69	2706	5.0	15.3	5.69	63.5	53.9	45.0	87.7
7.5	148	7.4	1.09	24.4	17.2	195/139	138	24.1	3.32	4071	7.5	24.4	7.35	64.4	58.3	48.5	
15	171	10.4	1.77	44.7	28.8	166/98	88	31.7	2.79	3984	12.0	22.8	8.17	75.7	67.8	50.0	22.7
25	161	8.8	1.41	36.3	37.1	168/108	89	30.0	2.64	3990	9.0	19.0	7.20	75.3	70.2	52.0	31.2
35	163	7.9	1.28	36.0	54.5	163/112	106	27.5	2.92	4041	6.0	18.1	6.20				
45	146	7.8	1.13	15.8	42.7	160/116	114	-	*	3891	4.0	17.7	*	75.4	58.8	53.0	**
55	144	7.9	1.13	15.5	44.9	165/123	119	21.4	2.55	3840	3.5	17.4	6.82				
65	143	8.0	1.14	15.3	42.3	171/130	122	17.2	2.10	3906	3.0	17.4	8.28				
75	143	8.5	1.21	14.9	42.5	168/130	124	17.5	2.17	3837	4.0	16.9	7.79				
85	163	7.8	1.27	14.7	40.7	175/137	130	14.8	1.92	4080	3.0	16.3	8.49	76.2	52.0	51.5	41.2
95	175	7.0	1.22	15.8	40.2	174/137	133	14.8	1.97	4149	3.0	15.6	7.92				
105	184	6.7	1.23	16.0	35.3	175/138	139	14.0	1.94	4131	2.0	14.9	7.68				
115	161	5.8	0.93	16.4	37.2	178/140	133	17.0	2.26	4119	3.0	15.8	6.99	73.1	56.8	49.0	37.7
125	171	5.8	0.99	15.8	37.2	171/137	130	16.2	2.11	3720	2.5	17.3	8.20				
135	184	5.4	0.99	15,3	36.0	177/141	131	14.2	1.86	3816	3.0	17.7	9.52				

DRUG - Pyridostigmine

DOSE - 5.0 mg/kg

WEIGHT - 11.3 kg

DATE - 8/9/85

*equipment malfunctioning
**sample tube broken

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACh	P-R
-15	454	3.8	1.72	77.3	1.1	168/138	149	12.1	1.81	1518	2.0	11.7	6.46	76.7	47.1	42	256	82.0
.0	451	3.7	1.66	78.3	1.1	178/138	152	13.6	2.06	1701	3.5	12.4	6.02	53.9	46.0	44	272.1	82.0
7.5	273	4.9	1.33	54.1	2.8	181/128	105	19.5	2.05	1566	5.0	16.1	7.85	42.7	42.9	50.5		97.0
15	319	6.8	2.16	69.0	12.2	169/102	68	24.3	1.65	1539	7.0	15.2	9.21	53.5	44.6	52.5	95.6	108.0
25	295	8.0	2.36	65.5	32.5	158/109	75	19.3	1.45	1521	4.0	13.2	9.10	50.0	44.2	55.0	147.1	95.0
35	341	8.4	2.86	59.5	35.8	145/106	80	15.1	1.21	1407	4.0	11.4	9.42					89.0
45	355	8.5	3.01	75.5	25.9	141/105	78	14.7	1.15	1350	3.5	11.4	9.91	51.0	47.5	53.0	153.3	83.0
55	379	7.7	2.91	69.7	16.5	144/102	80	18.1	1.45	1347	3.5	12.5	8.62					83.0
65	415	7.3	3.02	54.5	15.7	143/102	84	14.9	1.25	1359	4.0	12.2	9.76					83.0
75	418	7.6	3.17	48.3	15.4	141/102	86	17.6	1.51	1350	4.0	11.8	7.81					83.0
85	389	8.7	3.38	55.7	16.0	137/102	88	13.9	1.22	1296	6.0	8.2	6.72	65.3	55.2	55.0	144.0	85.0
95	366	8.2	3.00	66.3	11.0	133/93	83	16.4	1.36	1158	6.0	8.4	6.18					87.0
05	403	6.7	2.70	53.8	8.0	151/95	73	21.4	1.56	1302	2.0	12.2	7.82					84.0
15	376	7.4	2.78	55.5	10.6	148/93	72	20.3	1.46	1293	2.0	10.9	7.46	51.7	47.3	54.0	137.6	84.0
25	443	7.7	3.41	62.9	5.7	140/87	70	18.4	1.29	1227	1.0	10.2	7.91					76.5
35	442	7.6	3.35	65.1	6.9	142/90	73	22.3	1.63	1263	4.0	10.3	6.32					76.5

DRUG - Pyridostigmine DOSE - 5.0 mg/kg * Equipment malfunctioning

WEIGHT - 11.1 kg DATE - 8/26/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	P-R
-15	510	6.9	3.51	88.1	1.5	136/96	102	17.5	1.78	1305	1.5	10.6	5.96	71.3	41.9	39.0	275.2	103.9
0	400	7.2	2.88	73.6	2.9	130/88	99	19.4	1.92	1233	3.0	10.3	5.36	67.4	46.3	39.0	273.5	105.8
7.5	231	14.8	3.41	38.9	13.1	155/101	92	21.0	1.93	1182	8.0	15.0	7.77	63.6	46.8	*		126.4
15	215	13.9	2.98	43.5	10.3	173/91	72	22.4	1.61	1617	13.5	18.1	11.24	55.1	44.8	52.0	91.9	116.4
25	273	20.8	5.67	26.8	24.6	156/80	59	27.5	1.62	1803	6.5	13.1	8.09	53.0	48.8	52.5	137.0	97.2
35	277	18.9	5.23	22.0	13.9	131/69	64	23.9	1.53	1632	3.5	11.3	7.38					92.4
45	291	15.3	4.45	28.0	10.8	120/63	67	20.7	1.39	1464	3.0	10.2	7.34	69.2	51.7	52.0	165.7	103.
55	267	15.7	4.19	29.4	18.5	120/65	68	21.3	1.45	1335	4.0	9.6	6.62					96.4
65	262	17.3	4.53	26.1	21.7	120/66	69	22.3	1.54	1314	3.5	9.6	6.23					103.4
75	270	16.3	4.40	27.6	17.7	120/69	71	19.1	1.36	1341	2.5	8.0	5.88					103.4
85	288	15.9	4.57	28.4	16.0	122/73	74	19.7	1.46	1341	3.0	7.6	5.20	73.3	38.4	49.0	160.9	104.4
95	298	16.7	4.97	30.6	16.1	115/70	81	17.5	1.42	1329	2.5	6.6	4.65					103.
105	311	21.4	6.65	30.9	16.3	110/69	90	15.1	1.36	1362	2.5	6.0	4.41					97.
115	282	13.9	3.91	46.6	11.7	107/66	89	16.1	1.43	1245	2.5	5.3	3.71	76.0	40.3	49.0	162.8	101.
125	286	14.9	4.26	39.4	13.5	108/67	91	13.4	1.22	1212	2.5	5.1	4.18					101.4
135	300	15.5	4.65	36.3	13.9	112/72	90	16.8	1.51	1233	1.5	5.6	3.71					93.

DRUG - Pyridostigmine DOSE - 5.0 mg/kg * Hct tube broken

WEIGHT - 11.2 kg DATE - 9/24/85

IME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	AChe	P-
-15	308	5.2	1.60	32.5	7.9	140/105	156	16.7	2.61	1185	3.5	15.7	6.01	63.9	29.7	*	248.4	74
0	322	4.7	1.51	33.6	8.6	138/105	145	16.9	2.74	2415	1.0	10.1	3.69	46.4	26.8	*	259.8	74
7.5	240	10.4	2.49	34.5	8.4	83/50	106	35.2	3.73	2592	6.5	15.2	4.08	34.4	30.9	*		88
15	287	9.9	2.84	25.9	10.4	175/95	51	48.8	2.49	2796	7.5	8.8	3.53	44.7	89.7	*	129.6	75
25	287	7.3	2.09	19.9	2.6	138/89	75	21.0	1.55	2076	5.0	8.9	5.74	59.3	49.7	*	181.4	98
35	243	10.8	2.62	17.2	27.3	107/62	76	20.9	1.59	1599	2.5	9.2	5.79					95
45	218	8.5	1.85	18.0	18.6	115/73	84	20.8	1.75	1839	2.0	7.4	4.23	65.9	53.5	*	155.5	92
55	214	10.0	2.14	17.9	21.6	134/86	93	19.1	1.78	2043	2.0	7.6	4.27					91
65	356	10.0	3.56	63.8	15.3	131/84	96	17.5	1.68	2235	2.5	7.2	4.29					95
75	335	10.1	3.38	52.3	6.1	131/85	97	16.2	1.57	2313	4.0	7.0	4.46					94
85	282	10.9	3.07	35.2	4.3	130/87	102	17.6	1.80	2541	2.0	6.5	3.61	74.6	53.6	*	147.8	94
95	246	10.0	2.46	27.9	2.1	130/88	106	17.6	1.87	2223	3.5	6.2	3.32					91
105	223	8.1	1.80	24.9	8.6	131/89	106	15.6	1.65	2619	2.5	8.6	5.21					84
115	262	6.7	1.75	28.8	15.6	133/92	110	15.4	1.69	2715	1.5	9.3	5.50	70.3	52.5	*	199.7	86
125	227	9.5	2.15	26.2	18.2	131/94	120	14.8	1.78	2763	1.0	8.8	4.94					84
135	252	9.3	2.34	26.4	12.9	130/94	120	12.8	1.54	2688	2.0	9.3	6.04					79

DRUG - Pyridostigmine DOSE - 5.0 mg/kg * Hct tube broken

WEIGHT - 14.2 kg DATE - 10/2/85

TIME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACbc	
-15	118	12.9	1.52	28.7	3.4	175/132	159	11.4	1.81	3150	5.5	10.8	5.97	87.8	60.9	39.5	254.2	8
0	106	17.2	1.82	28.7	3.2	180/140	162	11.0	1.78	2850	5.0	8.2	4.61	90.0	51.5	40.0	251.9	8
7.5	73	18.2	1.32	18.5	34.5	175/135	129	16.1	2.08	2832	2.5	8.9	4.28	57.9	54.0	46.0		10
15	55	16.4	0.90	14.6	39.3	175/75	76	25.1	1.91	3498	8.5	18.0	9.42	86.4	67.8	54.5	††	10
25	103	25.1	2.58	15.0	50.1	155/80	76	17.4	1.32	†	9.0	11.0	8.33	*	*	*	*	10
35	116	23.2	2.69	15.3	44.2	135/70	77	17.0	1.31	2712	8.0	9.0	6.87					10
45	102	23.9	2.43	11.3	40.4	140/80	78	21.3	1.66	2737	5.0	8.7	5.24	58.9	49.5	57.5	141.1	10
55	102	26.0	2.65	11.3	39.9	125/70	79	18.5	1.46	2694	4.0	4.3	2.94					10
65	76	28.7	2.18	10.1	48.6	130/70	81	17.7	1.43	2604	**	2.8	1.96					10
75	71	23.9	1.69	12.0	38.8	135/70	80	16.5	1.32	2454	**	1.6	1.21					10
85	76	25.7	1.95	11.4	40.0	143/92	81	14.9	1.21	2382	6.0	8.7	7.19	82.1	62.1	56.0	133.5	11
95	75	27.2	2.04	11.1	43.5	140/89	81	14.8	1.20	2337	7.0	8.6	7.17					11
105	73	29.0	2.11	11.1	36.8	145/95	84	13.2	1.11	2400	5.0	8.4	7.57					11
115	66	24.1	1.59	14.2	31.5	149/93	84	14.2	1.19	2367	2.5	5.0	4.20	82.3	59.3	55.5	153.9	10
125	64	22.1	1.41	13.7	39.2	152/94	85	13.4	1.14	2583	2.0	8.8	7.72					10
135	67	22.9	1.53	12.4	42.0	154/99	87	12.2	1.06	2598	6.0	8.1	7.64					10

DRUG - Pyridostigmine

DOSE - 5.0 mg/kg

WEIGHT - 11.5kg

DATE - 10/23/85

*Arterial and renous blood sample tubes brok
†Miller® catheter position moved
**Equipment malfunction
††Blood sample tube lost

IME	TV	RESP RATE	MV	C	R	ABP	HR	SV	CO	dP/dt	PWP	PAP	PVR	aPo_2	vPo_2	Hct	ACha	. P-
15	175	13.5	2.36	27.4	8.3	180/115	149	17.2	2.57	2331	3.5	11.3	4.40	73.7	47.3	**	213.2	83.5
.0	170	14.5	2.46	26.2	8.8	180/115	152	17.2	2.61	2358	3.5	12.7	4.87	70.1	46.6	40.0	242.2	82.0
.5	151	17.5	2.64	52.9	12.6	210/125	128	22.0	2.81	2874	7.5	15.0	5.34	*	*	50.0		95.0
15	164	17.7	2.90	23.4	14.5	175/100	98	24.7	2.42	2925	8.5	13.7	5.66	82.6	49.4	52.0	71.8	95.0
25	150	18.1	2.71	19.6	22.3	160/100	102	18.8	1.92	2388	5.0	9.8	5.10	73.5	44.1	51.0	101.5	103.5
35	173	17.1	2.95	21.4	21.3	165/100	101	19.2	1.94	2277	5.0	8.8	4.54					101.0
45	150	15.8	2.37	20.4	22.2	160/100	103	17.7	1.82	1926	4.5	9.1	5.00	70.0	46.7	43.0	102.9	102.5
55	151	17.3	2.61	20.2	19.4	155/100	106	14.9	1.58	1974	5.0	9.0	5.70					100.0
65	151	15.6	2.35	23.7	19.3	170/110	106	16.3	1.73	1992	4.5	8.7	5.03					96.5
75	183	14.4	2.63	24.3	16.8	160/100	111	14.5	1.61	1956	5.0	7.7	4.78					97.5
85	175	14.0	2.45	22.7	17.8	170/110	110	14.5	1.60	1947	4.5	8.2	5.13	77.1	43.8	40.5	66.3	97.0
95	170	15.0	2.55	21.9	15.9	165/105	114	12.9	1.47	1938	2.0	8.8	5.99					95.0
05	173	13.5	2.33	23.1	14.9	165/105	116	12.3	1.48	1872	5.0	8.4	5.68					92.0
15	175	13.8	2.41	22.6	14.2	165/105	114	13.3	1.52	1896	7.0	8.5	5.59	77.8	42.8	44.0	95.3	92.0
25	175	15.1	2.64	24.0	12.0	165/105	119	12.6	1.50	1896	6.5	8.6	5.73					89.0
35	173	15.8	2.73	23.1	13.2	165/110	123	12.5	1.54	1956	5.5	8.7	5.65					89.0

DRUG - Pyridostigmine

DOSE - 5.0 mg/kg

*Arterial and Venous blood sample tubes broken
**Hct tube broken

WEIGHT - 12.1 kg

DATE - 11/19/85

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